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

OCT 0 3 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Chlorpyrifos - Review of a Two-Generation Reproduction Study in Rats (PC Code: 059101; DP Barcode: D166124; Caswell No. 219AA)

FROM: Elizabeth A. Doyle, Ph.D., Section Head
Review Section IV, Tox Branch II (H7509C)

EAD Sep 6
9/21/92

TO: Carl Andreasen, PM-19
Registration Division (H7505C)

THRU: Marcia van Gemert, Ph.D., Chief
Toxicology Branch II
Health Effects Division (H7509C)

MvanGemert
9/21/92

Registrant: DowElanco

Action Requested: Review of a two-generation reproduction study of chlorpyrifos in rats (MRID 419303-01).

Summary: Male and female Sprague-Dawley rats were fed diets containing 0, 0.1, 1.0, or 5.0 mg chlorpyrifos/kg BW/day for 10 or 12 weeks prior to mating (F_0 or F_1 generation, respectively, with exposure continuing through lactation and weaning. Cholinesterase inhibition in brain, plasma and red blood cells was observed in parental animals at treatment levels of 1.0 and 5.0 mg/kg/day. Histological lesions of the adrenal gland were reported in parental rats at 5.0 mg/kg/day.

Neonatal effects consisted of reduced pup weights and increased mortality at 5.0 mg/kg/day.

Developmental NOEL = 1.0 mg/kg/day
Developmental LOEL = 5.0 mg/kg/day based upon reduced pup weights and increased mortality

Parental NOEL = 0.1 mg/kg/day
Parental LOEL = 1.0 mg/kg/day based upon cholinesterase inhibition

This study was classified as Core - Guideline and satisfies the guideline requirements (83-4) for a Two-Generation Reproduction Study in Rats.

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FINAL

DATA EVALUATION REPORT

CHLORPYRIFOS

A Two-Generation Reproduction Study in Rats

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
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Contract Number: 68D10075
Work Assignment Number: 1-17
Clement Number: 91-94
Project Officer: James Scott

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Guideline No. 83-4;
Reproductive Toxicity

EPA Reviewer and
Section Head: Elizabeth Doyle, Ph.D.
Review Section IV, Toxicology Branch II/HED

Signature E. A. Doyle
Date 12/17/91

DATA EVALUATION REPORT

STUDY TYPE: Reproductive Toxicity

EPA IDENTIFICATION NUMBERS:

Tox Chem. Number: 219AA

MRID Number: 419303-01

HED Project Number: 1-1698

TEST MATERIAL: Chlorpyrifos technical; O-O-dimethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate

SYNONYMS: Brodan; Eradex; Piridane; Dursban

SPONSOR: DowElanco, 9002 Purdue Road, Indianapolis, Indiana 46268-1189

STUDY NUMBER: K-044793-088

TESTING FACILITY: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, Michigan 48674

TITLE OF REPORT: Chlorpyrifos: Two-Generation Dietary Reproduction Study in Sprague-Dawley Rats

AUTHORS: Breslin, W.J., Liberacki, A.B., Dittenber, D.A., Brzak, K.A., and Quast, J.F.

REPORT ISSUED: June 5, 1991

CONCLUSIONS: In a two-generation reproduction study, male and female Sprague-Dawley rats were fed diets containing chlorpyrifos technical at 0, 0.1, 1.0, or 5.0 mg/kg/day. Parental toxicity was observed at 1.0 and 5.0 mg/kg/day as indicated by significant reductions in the cholinesterase activities of brain, plasma, and red blood cells. Histological lesions in the adrenal glands were noted in males and females receiving 5.0 mg/kg/day. Based on these results, the NOEL and LOEL for parental toxicity were 0.1 and 1.0 mg/kg/day, respectively. Neonatal effects (decreased pup body weight and increased pup mortality) were observed at 5.0 mg/kg/day which was also toxic to maternal animals. Therefore, the LOEL for neonatal effects was 5.0 mg/kg/day; the NOEL was 1 mg/kg/day.

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CORE CLASSIFICATION: CORE Guideline. This study meets the requirements set forth under Guideline 83-4 for a two-generation reproductive toxicity study in rats. Although several minor study deficiencies were noted, they did not affect the integrity of the test results.

A. MATERIALS

Test Compound

Purity: 97.8-98.5%.
Specific gravity: 1.398 at 43.5°C
Description: whitish-tan granular crystals
Lot no.: not reported
Receipt date: not reported
Other provided information: MW: 350.6; melting point: 41.5-43.5°C
Contaminants: not reported
Stability: at least 51 days

Vehicle(s): None used. The test material was administered in the diet.

Test Animal(s)

Species: rat
Strain: Sprague-Dawley
Source: Charles River Breeding Laboratory, Portage, MI
Age: approximately 6 weeks of age at initiation of dosing
Body weight: Prior to initiation of treatment, males weighed 114.6-148.5 g and females weighed 95.6-111.2 g.

B. STUDY DESIGN

This study was designed to assess the potential of chlorpyrifos technical to cause reproductive toxicity when administered continuously in the diet for two successive generations. Rats were acclimated to laboratory conditions for at least 1 week.

Animal Husbandry: Animals were housed individually in wire-mesh, stainless-steel cages. During the gestation/lactation phases of the study, females were housed in plastic cages provided with ground corn cob nesting material. Basal diet and tap water were provided ad libitum.

Mating Procedure: The F_0 and F_1 parental animals were mated after 10 and 12 weeks, respectively, of dietary treatment. Each breeding regimen was comprised of three 7-day cohabitation periods with one female and one male of the respective treatment groups. Vaginal lavage samples were examined daily for the presence of sperm. The day the sperm was detected was designated gestation day 0. Sibling matings were avoided. Females failing to mate during the first 7-day mating period were placed with an alternate male from the same treatment group for the second 7-day period; the same procedure was followed for the third 7-day period.

Group Arrangement: F_0 animals were randomly assigned by body weight, and F_1 parental animals were assigned (method not specified) to groups as follows:

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group			
		F0		F1	
		Males	Females	Males	Females
Control	0	30	30	30	30
Low dose	0.1	30	30	30	30
Mid dose	1.0	30	30	30	30
High dose	5.0	30	30	30	30

Dose Administered: The test material was administered continuously in the diet for two consecutive generations. The test diets were prepared weekly by making several dilutions of premixes (mixtures of test material and rodent chow). The concentration of the test material in the diets was calculated from weekly body weights and food consumption data in order to maintain the targeted dose levels on a mg/kg/day basis. Animals received the appropriate diets for about 10 and 12 weeks of treatment prior to breeding of the F_0 and F_1 generations, respectively. Dietary concentrations were adjusted during lactation based upon historical food consumption data for lactating females.

The homogeneity of the test material in the 0.1 mg/kg/day dietary preparation was determined on the basis of single sampling from the top, middle, and bottom portions of three separate core diets. The stability of the 0.1-mg/kg/day test diet was determined at days 0, 3, 14, and 51. Concentration analyses of the test material in the diet were conducted on samples from each dose level at least three times per generation. Dose levels were selected based on the findings of previously conducted 2- and 13-week dietary studies, a chronic toxicity/oncongenicity study, and two reproduction studies in rats. From the results of the oncogenicity and reproduction studies, it was anticipated that the high dose would decrease brain, plasma, and red blood cell cholinesterase activities.

Observations: Animals were observed at least once a day for overt signs of toxicity. Body weights and food consumption for all animals were determined weekly prior to breeding. Male body weights were recorded weekly throughout the duration of the study: sperm positive females were weighed on days 0, 7, 14, and 21 of gestation. Females that delivered litters were weighed on days 1, 4, 7, 14, and 21 of lactation. At the end of the breeding periods, food consumption was measured weekly in males. Food consumption was measured at weekly intervals in sperm positive females during gestation. After parturition, food consumption was measured once during the first week of lactation, twice during the second week of lactation, and at 2-3 day intervals during the last week of lactation. A comparable protocol was utilized for the F_1 generation.

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The following data were recorded for each litter:

- the date of parturition;
- litter size on the day of parturition (day 0);
- the number of live and dead pups on days 0, 1, 4, 7, 14, and 21 postparturition;
- sex and weight of each pup on days 1, 4 (before and after culling), 7, 14, and 21 of lactation.

Obvious physical or behavioral abnormalities in the neonates were recorded during the lactation period.

Plasma, erythrocyte, and brain cholinesterase activity were determined for the first 10 F_0 and F_1 adult rats/sex/dose at the scheduled necropsy. Blood samples were collected from the orbital sinus for plasma and red blood cell cholinesterase determinations.

All F_0 and F_1 adults, including those found dead, were necropsied; selected organs were weighed and tissues were preserved. The scheduled necropsy was conducted after the last litter of the respective generation had been weaned. The following tissues were collected from the control and high-dose animals for histopathological examination:

- Adrenals
- Brain
- Cervix
- Coagulating Glands
- Epididymides
- Gross Lesions
- Ovaries
- Oviducts
- Pituitary
- Prostate
- Seminal Vesicles
- Testes
- Uterus
- Vagina

Also, the livers of 10 control and 10 high-dose F_1 adults males were examined.

At weaning, gross pathological examinations were performed on 10 pups/sex/dose level from the F_1 and F_2 litters selected at random. Terminal body weights were not recorded, and histologic examinations of tissues was not performed.

Statistical Analysis: Body weights, body weight gains, and plasma, red blood cell, and brain cholinesterase data were evaluated by Bartlett's test for equality of variances. Further analysis was performed by ANOVA, Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction.

Compliance:

- A signed Statement of Compliance with Good Laboratory Practice Standards, dated June 5, 1991 and May 23 1991, was provided;
- A signed Quality Assurance Statement, dated June 4, 1991, was provided; and
- A signed Statement of No Data Confidentiality Claim, dated May 23, 1991, was provided.

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C. RESULTS

1. Test Material Analysis: Concentrations of the test material in the diets ranged from 93% to 100% of nominal values. Homogeneity analysis of the 0.1-mg/kg/day test diet (based on a single determination for the top, middle, and bottom portions of three separate core samples) revealed a concentration of 99% (CV = 2.9%) of nominal value. Analyses for stability of the test material in the 0.1-mg/kg/day diet at days 0, 3, 14, and 51 indicated concentrations of 93%, 101%, 96%, and 96% of nominal values, respectively.

2. Parental Toxicity

Mortality: No treatment-related mortalities were observed. One control F_0 male was found dead on test day 125, 7 days prior to the scheduled necropsy. The cause of death could not be determined; however, there were no abnormal clinical signs noted in this male prior to its death. Gross pathological examination of this rat revealed perineal soiling, blood around the muzzle, and general visceral congestion.

One F_1 male receiving 0.1 mg/kg/day was found dead on test day 66; no remarkable clinical signs were observed. Bilateral renal and urinary calculi were noted at the gross pathological examination. The kidneys were enlarged with a thin cortex and contained foul-smelling urine. The study authors attributed the cause of death to renal and bladder calculi.

Clinical Observations: No treatment-related effects were observed in either F_0 or F_1 animals at any dose level during the exposure period.

Body Weight: Mean body weights of all treated F_0 males were comparable to those of controls throughout the study. Mean body weights of all treated F_0 females were also similar to those of controls during premating (Table 1), gestation (Table 2), and lactation periods (Table 3). Total body weight gains in the high-dose F_0 females were slightly but significantly ($p \leq 0.05$) lower than control during lactation days 1-21. The study authors attributed the decrease in female body weight gain to the combined stress of treatment and lactation. Also, the decrease in body weight gain in the high-dose females corresponded to the slight decreases in food consumption during the last 2 weeks of lactation. No compound-related effects on body weight gain were seen in high-dose F_0 females during gestation or in low- or mid-dose F_0 females during lactation.

Slight but not significant reductions in the body weight and body weight gain of adult F_1 males were observed throughout the study. There were no treatment-related effects on body weight or body weight gain of treated F_1 females during the premating, gestation, or lactation periods.

Food Consumption: Food consumption in all treated F_0 males was similar to that of controls throughout the study. Food consumption in all treated F_0 females was comparable to that of controls during the premating and gestation periods. However, during the last 2 weeks of

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lactation, food consumption for the high-dose F_0 dams was lower (about 7-11%) than controls.

There were no significant treatment-related effects at any dose level on food consumption in F_1 males throughout the study period or in F_1 females during the premating, gestation, or lactation periods.

Gross and Microscopic Pathology: No treatment-related gross pathological changes were observed in either F_0 or F_1 male or female adults at any dose level.

Increased incidence of histologic findings was observed in the adrenal glands of both F_0 and F_1 adult females receiving 5.0 mg/kg/day (Table 4). Histological alterations in the adrenal gland were confined to the cells of the zona fasciculata and consisted of very slight to slight vacuolation. These findings were consistent with very slight tinctorial properties in females.

Clinical Chemistry: Significant ($p \leq 0.05$) dose-related decreases in the plasma and erythrocyte cholinesterase activities were noted in the mid- and high-dose F_0 and F_1 male and female adult rats (Table 5). Significant ($p \leq 0.05$) inhibition of brain cholinesterase activity was also noted in F_0 adult males (52% of control values) and F_0 adult females (51% of control values) receiving the high dose. Significant ($p \leq 0.05$) decreases in brain cholinesterase activity were also observed in the high-dose F_1 animals. For these groups, brain cholinesterase activity in males and females was 47% and 42% of controls, respectively.

Table 1: Summary of Body Weights During the Premating Period for Rats Fed Chlorpyrifos for Two Successive Generations[a]

Dose Levels (mg/kg/day)	Mean Body Weight (g ± SD) on Study Day: -2	20	41	69
F_0 Males				
0	193.2 ± 12.0	340.7 ± 25.2	410.8 ± 32.5	479.6 ± 42.5
0.1	192.2 ± 12.9	341.3 ± 31.0	410.3 ± 38.2	482.0 ± 49.5
1.0	192.6 ± 10.8	338.1 ± 20.6	407.9 ± 27.8	476.5 ± 38.9
5.0	191.9 ± 13.2	335.3 ± 27.2	401.8 ± 29.6	471.7 ± 35.9
F_0 Females				
0	137.6 ± 6.0	198.3 ± 14.9	226.7 ± 20.4	258.1 ± 26.5
0.1	137.1 ± 5.7	197.8 ± 13.7	226.5 ± 15.0	253.6 ± 17.7
1.0	135.0 ± 6.0	193.3 ± 11.8	223.2 ± 15.3	251.2 ± 20.7
5.0	138.1 ± 6.0	195.7 ± 15.4	225.9 ± 19.2	257.5 ± 24.3
F_1 Males				
0	148.9 ± 17.4	326.7 ± 32.4	423.2 ± 40.5	511.4 ± 52.6
0.1	150.4 ± 18.9	317.8 ± 29.0	407.8 ± 37.8	496.5 ± 49.2
1.0	144.2 ± 24.6	315.4 ± 36.2	409.7 ± 33.5	491.7 ± 43.4
5.0	141.4 ± 18.0	308.5 ± 35.2	398.5 ± 45.7	482.0 ± 60.0
F_1 Females				
0	122.8 ± 13.6	195.4 ± 15.9	232.0 ± 18.1	266.3 ± 25.8
0.1	126.4 ± 18.0	202.5 ± 22.7	239.5 ± 26.8	277.1 ± 31.7
1.0	117.3 ± 16.4	189.2 ± 17.5	224.7 ± 19.9	258.1 ± 22.6
5.0	117.1 ± 10.5	192.5 ± 16.6	228.7 ± 22.7	264.8 ± 26.4

[a]Data extracted from Study No. K-044793-088. Tables 11, 12, 31, and 32.

Table 2. Summary of Maternal Body Weight During Gestation in Rats Fed Chlorpyrifos for Two Successive Generations[a]

Dose Levels (mg/kg/day)	Mean Body Weight (g ± SD) on Gestation Day: 0	7	14	21
F_0 Females				
0	260.6 ± 25.6	286.7 ± 23.9	310.3 ± 23.4	386.6 ± 27.7
0.1	254.8 ± 18.0	280.8 ± 17.4	309.1 ± 19.7	392.3 ± 26.7
1.0	251.3 ± 18.7	275.9 ± 15.5	303.9 ± 16.9	383.3 ± 21.8
5.0	258.6 ± 24.1	282.3 ± 22.4	309.6 ± 22.5	392.4 ± 28.1
F_1 Females				
0	270.6 ± 26.0	294.6 ± 26.3	323.1 ± 26.7	396.7 ± 37.4
0.1	286.4 ± 33.4	308.7 ± 31.5	334.8 ± 31.9	417.0 ± 43.7
1.0	269.1 ± 25.5	294.0 ± 24.3	320.1 ± 24.4	399.0 ± 36.2
5.0	274.8 ± 24.5	298.1 ± 23.6	326.3 ± 23.9	398.5 ± 31.8

[a]Data extracted from Study No. K-044793-088, Tables 13 and 33.

Table 3. Summary of Maternal Body Weight During Lactation in Rats Fed Chlorpyrifos Technical for Two Successive Generations[a]

Dose Levels (mg/kg/day)	Mean Body Weight (g ± SD) on Gestation Day: 1	7	14	21
F_0 Females				
0	290.4 ± 19.3	303.7 ± 21.8	328.3 ± 21.6	322.4 ± 20.0
0.1	291.7 ± 20.9	309.4 ± 21.7	332.5 ± 24.0	326.5 ± 20.7
1.0	287.0 ± 18.0	300.5 ± 18.4	321.9 ± 19.0	313.9 ± 18.0
5.0	293.5 ± 20.5	302.2 ± 21.0	323.8 ± 22.9	317.5 ± 24.0
F_1 Females				
0	300.4 ± 25.7	317.2 ± 21.6	330.7 ± 24.2	317.1 ± 23.0
0.1-	311.9 ± 30.0	327.0 ± 24.5	340.3 ± 24.8	328.1 ± 24.4
1.0	297.7 ± 27.6	311.7 ± 26.4	326.8 ± 25.8	316.4 ± 23.7
5.0	305.3 ± 23.5	319.9 ± 23.4	335.5 ± 23.9	325.6 ± 21.4

[a]Data extracted from Study No. K-044793-088, Tables 14 and 16.

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Table 4. Incidences of Adrenal Gland Lesions in F_0 and F_1 Adult Rats Fed Chlorpyrifos For Two Successive Generations[a]

	Dose Level (mg/kg/day)							
	0		0.1		1.0		5.0	
	M	F	M	F	M	F	M	F
F_0 Generation								
Altered Tinctorial Properties, Zona Fasciculata	0	0	0	0	0	0	0	21
Vacuolation, very slight	14	0	7	0	12	0	19	16
Vacuolation, slight	1	0	1	0	0	0	5	0
F_1 Generation								
Altered Tinctorial Properties, Zona Fasciculata	0	2	0	1	0	1	0	18
Vacuolation, very slight	14	0	16	0	9	0	17	0
Vacuolation, slight	1	0	0	0	1	0	6	0

[a]Data were extracted from Study No. K-044793-088, Tables 20 and 40.

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Table 5. Cholinesterase Activities in F_0 and F_1 Rats Fed Chlorpyrifos for Two Successive Generations[a,b]

	Dose Level (mg/kg/day)			
	0	0.1	1.0	5.0
F_0 Males				
Red Blood Cell Cholinesterase (IU/ML)	1.08 ± 0.11	1.03 ± 0.12	0.33 ± 0.15*	0.32 ± 0.16*
Plasma Cholinesterase (IU/ML)	0.54 ± 0.16	0.46 ± 0.09	0.30 ± 0.03*	0.21 ± 0.03*
Brain Cholinesterase (IU/G)	9.33 ± 0.38	9.24 ± 0.42	8.75 ± 0.63	4.87 ± 0.71*
F_0 Females				
Red Blood Cell Cholinesterase (IU/ML)	1.02 ± 0.07	1.05 ± 0.17	0.36 ± 0.11*	0.31 ± 0.08*
Plasma Cholinesterase (IU/ML)	1.95 ± 0.70	1.55 ± 0.52	0.80 ± 0.36*	0.64 ± 0.16*
Brain Cholinesterase (IU/G)	8.98 ± 0.43	9.11 ± 0.25	8.73 ± 0.20	4.60 ± 0.61*
F_1 Males				
Red Blood Cell Cholinesterase (IU/ML)	1.13 ± 0.11	0.98 ± 0.13*	0.37 ± 0.07*	0.33 ± 0.04*
Plasma Cholinesterase (IU/ML)	0.53 ± 0.12	0.43 ± 0.08	0.30 ± 0.03*	0.19 ± 0.02*
Brain Cholinesterase (IU/ML)	9.70 ± 0.36	9.74 ± 0.42	9.36 ± 0.32	4.60 ± 0.94*
F_1 Females				
Red Blood Cell Cholinesterase (IU/ML)	0.96 ± 0.17	0.97 ± 0.08	0.32 ± 0.14*	0.24 ± 0.12*
Plasma Cholinesterase (IU/ML)	1.83 ± 0.57	1.55 ± 0.37	0.93 ± 0.26*	0.52 ± 0.11*
Brain Cholinesterase (IU/ML)	9.44 ± 0.54	9.20 ± 0.70	9.01 ± 0.64	3.98 ± 1.03*

[a]Data were extracted from Study No. K-044793-088, Tables 17, 18, 37, and 38.
[b]Cholinesterase activities were determined on the first 10 rats/sex/dose during the scheduled necropsy.

*Significantly different from controls ($p < 0.05$).

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3. Reproductive Toxicity: The effects of dietary administration of the test material on reproductive parameters in the first generation F_0 adults/F_1 litters are summarized in Table 8. No treatment-related effects were observed on the male or female fertility indices, length of gestation, time to mating, pup sex ratio, or litter size. The significant ($p \leq 0.05$) decreases in the female conception and fertility index noted in the low-dose females were regarded by the study authors to be incidental since these effects were absent in the mid- and high-dose females. Pup survival was significantly decreased on days 14 and 21 of lactation in the high-dose group. The decrease in pup survival occurred at a dose level associated with parental toxicity (i.e., significant decreases in blood and brain cholinesterase activity and histopathologic alterations of the adrenal zona fasciculata).

There were no remarkable clinical signs or physical alterations in any of the F_1 pups. A slight but nonsignificant reduction in body weight was seen in male and female F_1 pups from the high-dose group on day 0 of lactation; however, the body weights were significantly decreased on days 4, 7, 14, and 21 of lactation. The decrease in F_1 pup weights at the high dose corresponded to decreased pup survival and lower body weight gain of high-dose dams during lactation. Also, the reduced pup weights occurred at a dose level associated with parental toxicity. In general, findings for the lower doses were comparable to the controls.

No treatment-related effects were seen on reproductive parameters, such as fertility, length of gestation, time to mating. pup sex ratio, pup survival, or litter size, in the F_1 adults and F_2 litters (Table 9). However, pup survival in both the control and high-dose groups was lower than normal during lactation. The decreased pup survival resulted from the death of three entire litters (25 pups) in the control group and five entire litters (48 pups) in the high-dose group.

Nevertheless, survival of pups in the high-dose group was 15% lower than controls. The study authors attributed this decrease to the sizable number of high-dose dams that neglected their litters. Therefore, the decrease in pup survival in the high-dose group was not considered by the study authors to be treatment-related.

Table 8. Summary of Effects of Dietary Administration of Chlorpyrifos on F_0 Adult/F_1 Litter Reproductive Parameters, Offspring Survival, and Pup Body Weight[a]

Parameter	Dose Level (mg/kg/day)			
	0	0.1	1.0	5.0
No. of matings	30	30	30	30
No. of pregnancies	30	24	28	28
Fertility index-female (%)	100	80*	93.3	93.3
Gestation index (%)	100	100	100	100
Gestation length (days)	21.8	21.6	21.6	21.6
Total number of live pups				
Day 0	400 (30)[b]	326 (24)	366 (28)	388 (28)
Day 4 (precull)	388	301	360	360
Day 21	236	182	222	210
Mean number of live pups/litter				
Day 0	13.3	13.6	13.1	13.9
Day 4 (precull)	12.9	12.5	12.9	12.9
Day 21	7.9	7.6	7.9	7.5
Live birth index (%)[c]	98.3	99.1	99.5	98.7
Viability index (%)[d]	97.0	92.3	98.4	92.8
Lactation index (%)[e]	99.2	98.9	99.1	95.5*
Mean pup body weight/litter (g)				
Day 0, male	6.5	6.4	6.4	6.2
female	6.2	6.0	6.1	5.8
Day 4, male (precull)	8.5	8.7	8.4	7.6*
female (precull)	8.1	8.2	8.1	7.2*
Day 14, male	29.5	30.5	28.6	26.5*
female	28.3	29.1	27.6	25.3*
Day 21, male	48.4	49.1	46.1	43.0*
female	46.1	46.9	44.7	41.3*
Sex ratio on Day 1 (Male:Female)	54:46	50:50	49:51	51:49

[a]Data extracted from Study No. K-044793-088, Tables 22 and 23.

[b]Number of litters within parentheses

[c]Live birth index is calculated as: $\frac{\text{No. of live pups born}}{\text{No. of live and dead pups born}} \times 100$

[d]Viability index calculated as: $\frac{\text{No. of live pups alive on day 4 precull}}{\text{No. of live pups alive on day 0}} \times 100$

[e]Lactation index calculated as: $\frac{\text{No. of live pups alive on day 21 precull}}{\text{No. of live pups alive on day 4 postcull}} \times 100$

*Significantly different from control ($p \leq 0.05$).

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Table 9. Summary of Effects of Dietary Administration of Chlorpyrifos on F_1 Adult/F_2 Litter Reproductive Parameters, Offspring Survival, and Pup Body Weight[a]

Parameter	Dose Levels (mg/kg/day)			
	0	0.1	1.0	5.0
No. of matings	30	30	30	29
No. of pregnancies	24	23	26	22
Fertility index-female (%)	80.0	76.7	86.7	73.3
Gestation index (%)	100	100	100	100
Gestation length (days)	21.7	21.6	21.8	21.9
Total number of live pups				
Day 0	277 (24)[b]	295 (23)	322 (26)	261 (22)
Day 4 (precull)	250	283	303	211
Day 21	163	174	186	128
Mean number of live pups/litter				
Day 0	11.5	12.8	12.4	11.9
Day 4 (precull)	10.4	12.3	11.7	9.6
Day 21	6.8	7.6	7.2	5.8
Live birth index (%)[c]	96.9	97.4	99.4	99.6
Viability index (%)[d]	90.3	95.9	94.1	80.8
Lactation index (%)[e]	96.4	98.3	98.4	92.1
Mean pup body weight/litter (g)				
Day 0, male	6.3	6.6	6.3	6.3
female	5.9	6.1	5.9	5.9
Day 4, male (precull)	8.6	9.0	8.6	8.4
female (precull)	8.2	8.6	8.2	7.8
Day 14, male	28.2	28.4	27.4	26.7
female	25.8	27.8	26.3	25.1
Day 21, male	45.1	47.6	43.7	43.4
female	42.7	45.7	41.7	41.0
Sex ratio on Day 1 Male:Female	47:53	48:52	48:52	49:51

[a]Data extracted from Study No. K-044793-088, Tables 42 and 43.

[b] Number of litters within parenthesis

[c]Live birth index is calculated as: $\frac{\text{No. of live pups born}}{\text{No. of live and dead pups born}} \times 100$

[d]Viability index calculated as: $\frac{\text{No. of live pups alive on day 4 precull}}{\text{No. of live pups alive on day 0}} \times 100$

[e]Lactation index calculated as: $\frac{\text{No. of live pups alive on day 21}}{\text{No. of live pups alive on day 4 postcull}} \times 100$

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D. REVIEWER'S DISCUSSION/CONCLUSIONS

1. Test Material Analyses: Results of the chemical analysis indicated that diets were accurately prepared. Similarly, the homogeneity and stability studies conducted in the 0.1 mg/kg dietary mixtures showed that the test material was evenly distributed throughout the feed and stable under the conditions of use. Concentrations of the test material in the diets ranged from 93% to 100% of nominal values.

2. Parental Toxicity: Treatment-related parental toxicity was observed at 1.0 and 5.0 mg/kg/day. Significant inhibition of plasma and red blood cell cholinesterase activity (25%-35%) was noted in F_0 and F_1 males and females receiving 1.0 and 5.0 mg/kg/day. In addition, the activity of brain cholinesterase was reduced by greater than 45% in the high-dose F_0 and F_1 males and females.

   Treatment-related histopathological lesions in the adrenal glands were seen in the high-dose F_0 and F_1 adult males and females. The histological changes were confined to the cells of the zona fasciculata and were characterized as very slight to slight vacuolation. Also, the histological changes in the adrenal gland were consistent with fatty changes in males and altered tinctorial properties in females. Moreover, the histological changes in the adrenal gland are consistent with findings in a previous subchronic 13-week dietary toxicity study in rats (Szabo et al. 1988) and a two-year dietary toxicity/oncogenicity study in rats (Young and Grandjean 1988).

   Mortality was observed in one adult low-dose F_0 male from the control group. The cause of death in this male was attributed by the study authors to renal and bladder calculi. The reviewers do not regard the death of this male to be indicative of a treatment-related effect since mortality was not observed at the higher dose levels.

   The body weights of adult F_1 males were slightly lower than controls throughout the study. There were no significant changes in food consumption in either F_0 or F_1 adults.

3. Reproductive Toxicity: Effects on the F_1 offspring occurred only at maternally toxic doses. F_1 pups from dams receiving 5.0 mg/kg/day exhibited decreased body weight and increased mortality. Although these effects were seen in F_2 litters. they were not statistically significant.

   There were no treatment-related effects on other reproductive parameters such as fertility indices, length of gestation, time to mating, pup sex ratio, pup survival, or litter size in either generation.

4. Study Deficiencies: Minor deficiencies noted are as follows:

   (a) Homogeneity of the test material in the diet was not examined at the 1.0- and 5.0-mg/kg/day dose levels.

   (b) No absolute and relative organ weights were reported.

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E. CORE CLASSIFICATION: Core Guideline Data

Parental Toxicity NOEL = 0.1 mg/kg/day

Parental Toxicity LOEL = 1.0 mg/kg/day based on cholinesterase inhibition

Reproductive/Developmental Toxicity NOEL = 1.0 mg/kg/day

Reproductive/Developmental Toxicity LOEL = 5.0 mg/kg/day based on decreased body weight and increased mortality

F. RISK ASSESSMENT: Not applicable

REFERENCES:

(1) Szabo, J.R., Young, J.T. and Grandjean, M. (1988). Chlorpyrifos: 13-Week Dietary Study in Fischer 344 Rats. Report of the Dow Chemical Company (unpublished study).

(2) Young, J.T. and Grandjean, M. (1988). Chlorpyrifos: Two-Year Dietary Toxicity-Oncogenicity Study in Fischer 344 Rats. Report of the Dow Chemical Company (unpublished study).

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Tox. Chem. No. 219AA (059101) File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD50, LC50, PIS, NOEL, LEL	Tox. Cat.	Core-Grade/ Doc. #
83-4 Two-Gen. Repro. in Rats K-044793-088 6/5/91	Chlorpyrifos technical	419303-01	Male and femal Sprague-Dawley rats were fed diets with 0, 0.1, 1.0 or 5.0 mg/kg/day for 10 (F0) or 12 (F1) weeks prior to mating, thru lactation and weaning. Cholinesterase inhibition in brain, plasma and RBC was observed in parental animals at 1.0 and 5.0 mg/kg/day. Adrenal lesions were reported in parental rats at 5.0 mg/kg/day. Neonatal effects consisted of reduced pup weights and increased mortality at 5.0 mg/kg/day. Develop. NOEL = 1.0 mg/kg/day; LOEL = 5.0 mg/kg/day based on reduced pup weights and increased mortality Parental NOEL = 0.1 mg/kg/day; LOEL = 1.0 based upon cholinesterase inhibition in brain, RBC and plasma		Guideline