CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

APR - 3 1989

007107

MEMORANDUM

SUBJECT: Chlorpyrifos - 2-Year Dietary Chronic Toxicity/ Oncogenicity Study - Rats

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

TO: Dennis Edwards
Product Manager (12)
Registration Division (H7505C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 3/23/89
Toxicology Branch II, Section II
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 3/23/89
Acting Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 3/30/89
Acting Chief, Toxicology Branch/HFAS/HED (H7509C)

Registrant: The Dow Chemical Company
Chemical: Chlorpyrifos; 0,0-diethyl-0-(3,5,6-trichloro-2-pyridinyl) phosphorothionate
Project: 9-0655
Caswell No.: 219AA
Record No.: 237765
Identifying No.: 464-404
MRID No.: 409528-02
Action Requested: Review data.

Comment: In response to the 1984 Chlorpyrifos Registration Standard, the above referenced long-term study in rats was submitted. Additionally, a 13-week feeding study on Chlorpyrifos was submitted under separate cover and it was reviewed elsewhere (MRID # 409528-01, HED Project No.: 9-0656, Record No.: 237763). The combined chronic/oncogenicity study has been reviewed and the DER is attached.

There were no significant differences in body weight, food consumption, or survival in either sex of rats feed Chlorpyrifos for two years at dose levels of 0.05, 0.1, 1, and 10 mg/kg/day. There was a consistent decrease in plasma cholinesterase activity in both sexes at the 1 and 10 mg/kg dose levels throughout the study. Brain cholinesterase was decreased at these same dose levels at 12 months, but only at 10 mg/kg (both sexes) at termination. However, the magnitude of the decrease at the 1 mg/kg dose level was less than 10% below control values. RBC cholinesterase activity was depressed at the 1 and 10 mg/kg dose levels in males throughout the study, although statistical significance was not attained at the 12-month time point, and the value in the 1 mg/kg males at termination was only 14% lower than the control value. There was no consistent decrease in RBC cholinesterase activity in females. Tumor incidence was comparable among the groups of both sexes. The NOEL can be set at 0.1 mg/kg; the LEL at 1 mg/kg, based on decreased plasma and brain cholinesterase activity.

007107

Reviewed by: Linda L. Taylor, Ph.D. Linda Taylor 3/22/89
Tox. Branch II, Section II, HED (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 3/23/89
Acting Section II Head, Tox. Branch, HED (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 104-week Chronic Toxicity/Oncogenicity-Rat TOX. CHEM. NO.: 219AA

MRID NO.: 409528-02

TEST MATERIAL: Chlorpyrifos - (0,0-diethyl-0-(3,5,6-trichloro-2-pyridinyl) phosphorothionate)

SYNONYMS: DOWCO*179, DURSBAN* insecticide, LORSBAN* insecticide

STUDY NUMBER: TXT:K-044793-079

SPONSOR: The Dow Chemical Company

TESTING FACILITY: Health and Environmental Sciences-Texas
Lake Jackson Research Center, The Dow Chemical Company

TITLE OF REPORT: Chlorpyrifos: 2-Year Dietary Chronic Toxicity-Oncogenicity Study in Fischer-344 Rats

AUTHOR: J.T. Young and M. Grandjean

REPORT ISSUED: December 23, 1988

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: There were no significant differences in body weight, food consumption, or survival in either sex of rats fed Chlorpyrifos for two years at dose levels of 0.05, 0.1, 1, and 10 mg/kg/day. There were decreases noted in erythrocyte and hemoglobin values, and an increase in platelets during the first year of the study in the 10 mg/kg dose group (both sexes), but these differences were not observed at termination. Urine specific gravity was increased also (10 mg/kg dose level) at various time points. There was a consistent decrease in plasma cholinesterase activity in both sexes at the 1 and 10 mg/kg dose levels throughout the study. Brain cholinesterase was decreased in both sexes at these same dose levels at 12 months (although the magnitude of the decrease at 1 mg/kg was less than 10% below control values), but only at 10 mg/kg at termination. Tumor incidence was comparable among the groups of both sexes. The NOEL can be set at 0.1 mg/kg; the LEL at 1 mg/kg, based on decreased plasma and brain cholinesterase activity.

Classification: Core minimum

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A. MATERIALS:

1. Test Compound: Chlorpyrifos, Description: white granular crystals, Batch #: AGR 214637, Purity: 98.5%.

2. Test Animals: Species: rat, Strain: Fischer 344, Age: 6 weeks, Weight: males: 117-118 grams, females: 96-97 grams, Source: Charles River Breeding Laboratory, Kingston, NY.

3. Statistics: A description of the various statistical analyses performed on the data is appended (Statistical Evaluation, pages 20 and 21 of the final report).

B. STUDY DESIGN:

1. Animal assignment

Rats (50/sex/group; housed individually) were administered Chlorpyrifos in the diet at dose levels of 0, 0.05, 0.1, 1, and 10 mg/kg body weight for 104 weeks. Ten additional rats/sex/group were randomly allocated for the 12-month sacrifice. The dose levels were chosen based on the subchronic study.

2. Diet preparation

Test diets were prepared once per week. Stability of the test material in rodent diet was previously determined (up to 42 days) and was not repeated for this study. Homogeneity checks were performed, which indicated a homogeneous dispersion of test material in the samples. Concentration of test material in the diets was measured pre-test and at 3-month intervals. The initial concentration of the test material was calculated from pre-test body weight and feed consumption. Thereafter, mean body weight and feed consumption were used to determine amount needed to maintain appropriate levels. The vehicle (if any) used for the control was not identified.

Results: The overall dietary concentrations were comparable to the targeted concentrations (85-124%).

3. Feed (Certified Rat Chow® #5002, Ralston Purian Co.) and water were provided ad libitum.

C. METHODS AND RESULTS:

1. Clinical Observations and Palpations

The animals were observed daily for signs of toxicity, abnormal behavior, mortality and morbidity, with a full examination being performed once weekly beginning after the sixth month. All animals were palpated for externally detectable masses during the pre-test period, prior to the 12-month sacrifice, and monthly thereafter until termination. "Palpable masses" were recorded , and the size, location, date of onset, progression/disappearance of each mass was

indicated.

Results:

Toxicity/Mortality (survival)

The number of animals dying on study is shown below.

CUMULATIVE MORTALITY

	MALES		FEMALES	
	18 months	24 months	18 months	24 months
mg/kg				
0.00	1	15	3	15
0.05	3	25	0	11
0.10	5	18	3	13
1.0	2	24	1	9
10.0	2	14	4	14

There was no treatment-related effect on survival. The common causes of death were listed as pituitary neoplasms and large granular lymphocyte leukemia (both expected in Fischer rats).

CAUSE OF DEATH (% incidence)*

	Pituitary adenoma/ adenocarcinoma		Large granular leukemia [first tumor - days on test]	
Dose level	Males	Females	Males	Females
0.0	47	33	13 [632]	20 [597]
0.05	20	27	16 [577]	36 [558]
0.1	30	15	28 [524]	62 [544]
1.0	38	22	21 [623]	44 [541]
10.0	36	21	36 [427]	50 [491]

* of those dying on study, the % with PA or LGL

2. Body Weight and Food Consumption

Body weight (individual) and food consumption (20 rats/sex/dose level) were determined weekly for the first 3 months and monthly thereafter.

Results: Body Weight

MALES - Body weight was statistically significantly lower in the high dose (10 mg/kg) from day 7 on (either by Dunnett's or Wilcoxon's test, alpha <0.05), and on days 42, 49, 84, 91, 147-231, 287-343, 399-511 at the 1 mg/kg level. It is to be noted that the difference from control never exceeded 9% for the 10 mg/kg animals and for the 1 mg/kg group, the body weight was 94-96% of control through day 90 and 91-93% of control from day 175 until termination. Overall body-weight gain in the 10 mg/kg group was approximately 90% of control.

FEMALES - Although body weight was also statistically significantly lower in the highest dose group compared with control (days 56-231), the values were 96-98% of control. Overall body-weight gains were comparable among the groups.

Food Consumption

The amount of food consumed was comparable among the groups for both sexes.

3. Blood Analysis

Clinical laboratory studies were conducted on all animals designated for the 12-month sacrifice at 6 and 12 months, and animals designated for terminal sacrifice were used for the 18-month and 2-year determinations (an effort was made to use the same animals at both latter time points).

a. Hematology: Blood samples were obtained from 10 fasted rats/sex/dose. The CHECKED (X) parameters were examined.

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)		Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)		Mean corpuscular volume (MCV)
X	Platelet count		Reticulocyte count

b. Clinical Chemistry: Blood samples were obtained from 10 fasted rats per sex/dose. The CHECKED (X) parameters were examined.

	Electrolytes:		Other:
X	Calcium*	X	Albumin*
X	Chloride*		Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Albumin/Globulin ratio
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Protein*
	Cholinesterase		Triglycerides
X	Creatinine phosphokinase*		Serum protein electrophoresis
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	gamma glutamyl transferase		
	glutamate dehydrogenase		

* Required for chronic studies

Results: HEMATOLOGY:

Males - At 6 months, the 10 mg/kg dose group displayed a decrease in total RBC, HGB, and PCV; at the 12-month sacrifice, a decrease in total RBC was still evident along with an increase in platelets. No other changes were reported.

Females - At 12 months, the 10 mg/kg dose group displayed decreases in RBC and HGB and an increase in platelets. The 0.1 and 1.0 mg/kg groups both displayed increases in PCV (not dose-related) at this same time point. No other differences were reported.

Note: There were no baseline data provided for any of the parameters measured.

CLINICAL CHEMISTRIES:

Males - Increased glucose values were observed at the 1 and 10 mg/kg dose levels at 6 months (not dose-related), and at the 0.05 mg/kg level at 18 months. Cholesterol, globulin, and total protein values were decreased at all time points in the 10 mg/kg dose level except at termination. Both the globulin (not dose-related) and total protein levels (dose-related) were also decreased at 18 months in the 0.1 and 1.0 mg/kg groups. Alkaline phosphatase was significantly lower at the 1 and 10 mg/kg dose levels (dose-related) at 12 months only. Sporadic differences in electrolytes occurred but none were consistent. Additionally, since two different sets of animals were used (one for the 6- and 12-month determinations and one for the 18-month and 2-year determinations), it is difficult to interpret these results. And as pointed out above, there were no baseline data.

Females - Increased glucose values were also observed at the 1 and 10 mg/kg dose levels at 6 months, which was not dose related, and at termination, the 10 mg/kg group displayed a significant decrease in glucose compared to control. Cholesterol values were decreased in the 1 and 10 mg/kg dose groups (dose-related), and the decrease continued in the 10 mg/kg group at the 12- and 18-month time points; the 1 mg/kg group displayed increased cholesterol levels at 12 months. Globulin values were decreased at the 10 mg/kg dose level at 6 and 12 months. Creatinine kinase values were significantly decreased (dose-related) at 18 months in the 0.1, 1.0, and 10 mg/kg dose groups. At termination, the 0.05 and 1 mg/kg dose groups displayed decreased values compared to control. Sporadic differences also occurred in electrolytes, but none were consistent.

4. Urinalysis

Urine was obtained about 1-2 weeks prior to the 12- and 24-month sacrifices and at 6 and 18 months from 10 rats/sex/dose level. The CHECKED (X) parameters were examined.

	Appearance*	X	Glucose*
	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin* (semiquantitative)
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen
	Osmolality		

*Required for chronic studies

Results

The specific gravity was increased (dose-related) in all test groups compared to their respective controls at all time points (3 exceptions) with statistical significance being attained only in the high-dose groups (both sexes at 6 months, in males at termination, and in females at 12 months). Since there were no baseline data, this difference may not be due to test material exposure, although the increases were observed in both sexes. The only other difference observed was in bilirubin values, with a tendency, especially in the females, for its occurrence with time. It is to be noted that the volume of urine was not measured (i.e., no values were reported).

5. Cholinesterase Activity

Cholinesterase activity (Photometric method - Boehringer Manneheim Diagnostics, Inc. 1981; modified for rodents) was assayed from the plasma and RBC's of 10 rats/sex/dose level (same animals as above). Brain cholinesterase activity was measured from a half-brain sample obtained after weighing at the 12-month (10 rats/sex/dose) and 24-month (20 rats/sex/dose) scheduled sacrifices.

Results

There was a dose-related (in most cases) decrease in cholinesterase activity (plasma, erythrocyte and brain) at each time point in both sexes.

MALES

Dose	6 months		12 months			18 months		24 months		
(mg/kg)	Plasma	Erythr.	Plasma	Erythr.	Brain	Plasma	Erythr.	Plasma	Erythr.	Brain
0	0.717	2.462	0.903	2.050	11.23	1.478	1.960	1.763	1.55	8.73
0.05	0.692	2.638	0.853	2.184	10.53*	1.380	2.136	1.629	1.63	8.90
0.10	0.682	2.186	0.883	1.910	10.46*	1.184	1.868	1.508	1.67	8.75
1	0.437†	1.874*	0.258†	1.382	10.22*	0.541†	1.298*	0.706†	1.34	8.97
10	0.317†	1.862*	0.120†	1.294	4.73*	0.333†	1.392*	0.348†	1.14*	3.87†

FEMALES

Dose	6 months		12 months			18 months		24 months		
(mg/kg)	Plasma	Erythr.	Plasma	Erythr.	Brain	Plasma	Erythr.	Plasma	Erythr.	Brain
0	3.683	1.730	4.138	1.964	11.75	3.919	1.882	2.854	2.19	8.86
0.05	3.605	1.614	4.296	1.732	11.50	3.884	2.146	2.945	2.35	8.92
0.1	3.351	1.838	3.600†	2.148	11.97	3.364†	1.876	2.693	1.91	8.86
1	1.275†	1.794	0.571†	1.614	11.20*	1.192†	1.468	1.133†	1.83	8.52
10	0.621†	1.514	0.198†	1.168†	4.57*	0.485†	1.542	0.510†	1.75	3.80†

* Dunnett's test; † Wilcoxon's test

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6. Gross Pathology: At the two scheduled sacrifices, the animals were necropsied after an overnight fast. An in situ examination of the eyes with a moist microscope slide under fluorescent light was performed. The brain, gonads, kidneys, liver, and adrenals were weighed and the relative weights (fasted body weight) were calculated.

Animals that died or were found moribund were necropsied similarly, but final body and organ weights were not measured. Additionally, the detailed eye examination was not performed on these latter rats.

The following CHECKED (X) organs/tissues were collected.

	Digestive system
X	Tongue
X	Salivary glands
X	Esophagus
X	Stomach
X	Duodenum
X	Jejunum
X	Ileum
X	Cecum
X	Colon
X	Rectum
X	Liver
	Gall bladder
X	Pancreas

	Respiratory
X	Trachea
X	Lung**
X	Nose
X	Pharynx
X	Larynx

	Cardiovasc./Hemat.
X	Aorta
X	Heart
X	Bone marrow
X	Lymph nodes*
X	Spleen
X	Thymus

	Urogenital
X	Kidneys
X	Urinary bladder
X	Testes
X	Epididymides
X	Prostate
X	Seminal vesicle
X	Ovaries
X	Uterus
X	Cervix
X	Oviduct
X	Vagina

	Neurologic
X	Brain (3 levels)
X	Periph. nerve (sciatic & tibial)
X	Spinal cord (3 levels)
X	Pituitary
X	Eyes

	Glandular
X	Adrenals
X	Lacrimal gland
X	Mammary gland
X	Parathyroids
X	Thyroids

	Other
X	Bone (femur)
X	Skeletal muscle
X	Skin
X	All gross lesions and masses
	Head+
X	Harderian gland
X	Coagulating gland
X	Oral tissue
X	Mesentric/mediastinal tissue

* mandibular and mesenteric (present did mediastinal, not mandibular)
** with mainstem bronchi
\+ 3 coronal sections including nasal cavity, paranasal sinuses, tongue, oral cavity, nasopharynx, and middle ear

Results:

In males, the adrenal weight (absolute and relative) was significantly increased over control values in the 10 mg/kg dose group at both sacrifice intervals. In females, the increase (both absolute and relative) occurred only at the terminal sacrifice. Relative brain weight was significantly increased at both sacrifices in the 10 mg/kg males and absolute brain weight was increased in the 10 mg/kg females only at terminal sacrifice. The absolute liver and kidney weights were decreased only at 24 months in the 10 mg/kg males, which reflected the lower body weights of this group. A comparable decrease was not observed in the females.

Gross pathological observations were comparable among the groups.

7. Histopathology: Representative sections of the tissues listed above were examined histologically for all rats in the control and 10 mg/kg dose groups at both sacrifice intervals. Additionally, all animals in the other dose groups that died during the study were examined. The tissues examined in the three low-dose groups at the 12-month sacrifice were the liver, kidneys, adrenal gland, and tissues with gross lesions. At the 24-month sacrifice, these latter four tissues plus the spleen, lungs, testes, pituitary, thyroid/parathyroid, and all significant gross lesions were examined in the three low-dose groups.

Results:

12-MONTH SACRIFICE

An excess of fatty vacuolization of the zona fasciculata in the adrenal cortex was observed in the 10 mg/kg dose males, which may account for the increase in adrenal weight seen at this sacrifice interval. It is noted that the control males at this age normally have some degree of mild vacuolization of the mid-adrenal cortex (recorded as very slight), but the females do not. The authors state that the increase observed in the 10 mg/kg males was an exacerbation of this vacuolization without obvious concommitant necrosis or cellular degeneration. There were no histopathological changes observed in the females at 12 months.

ADRENAL (male) Dose group (mg/kg)	0	0.05	0.1	1	10
N =	10	10	10	10	10
vacuolation consistent with fatty change, zona fasciculata - very slight	9	10	10	10	2
vacuolation consistent with fatty change, zona fasciculata - slight	1	0	0	0	8

ADRENAL (female) Dose group (mg/kg)	0	0.05	0.1	1	10
N =	50	50	50	50	50
vacuolation consistent with fatty change, zona fasciculata - very slight	0	0	0	0	0
vacuolation consistent with fatty change, zona fasciculata - slight	0	0	0	0	0

24-MONTH SACRIFICE

There was an increase in slight adrenal cortical vacuolization in the 10 mg/kg males similar to that seen at 12 months. Additionally, there was a decrease in the severity of chronic progressive glomerulonephropathy (chronic renal disease), a decrease in the incidence of mild biliary hyperplasia in the liver (also observed in the 10 mg/kg females), and a decrease in the incidence of hyperplasia in the mammary gland in

this male test group. In the spleen, it is noted that the 10 mg/kg females displayed a higher incidence (30%) of extramedullary hematopoiesis (moderate) than the other groups (12-18%), but a lower (0% vs 8%) incidence of extramedullary hematopoiesis (severe). Other statistically significant differences in histopathological lesions were observed in the 10 mg/kg group, but none could be related directly to test material administration.

There were no histopathological changes in any of the other dose groups in either sex that could be attributed to treatment. No pairwise increases or statistically identified linear trends were found in either sex at any dose level. The total number of tumors was comparable among the groups for each sex.

ADRENALS (males) Dose group (mg/kg)	0	0.05	0.1	1	10
N=	50	50	50	50	50
focus (i) of altered cells, cortex:	28	21	21	23	21
hyperplasia, medulla, diffuse:	1	3	0	3	3
necrosis, unilateral, diffuse:	0	0	0	0	0
necrosis, cortex, focal:	0	0	0	0	0
vacuolation consistent with fatty change, zona fasciculata - very slight	17	15	16	16	5
vacuolation consistent with fatty change, zona fasciculata - slight	2	5	4	5	20*
vacuolation consistent with fatty change, zona fasciculata - moderate	2	0	1	1	2

* alpha = 0.05, Yates Chi-squared test

ADRENALS (female) Dose group (mg/kg)	0	0.05	0.1	1	10
N=	50	50	50	50	50
focus (i) of altered cells, cortex:	30	28	24	37	38
hyperplasia, medulla, diffuse:	0	1	0	1	0
necrosis, unilateral, diffuse:	0	0	0	0	1
necrosis, cortex, focal:	1	1	1	0	0
vacuolation consistent with fatty change, zona fasciculata - very slight	3	0	2	0	2
vacuolation consistent with fatty change, zona fasciculata - slight	2	3	0	0	3
vacuolation consistent with fatty change, zona fasciculata - moderate	0	0	1	0	2

CONCLUSION

The administration of 10 mg Chlorpyrifos/kg body weight/day for two years to male rats resulted in a decrease in body weight gain compared to control, a depression of plasma, RBC, and brain cholinesterase, and an increase in the size of the adrenal gland characterized microscopically by increased fatty vacuolation of the zona fasciculata. Additionally, there were decreases observed in serum cholesterol, total protein and globulin, an

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increase in urine specific gravity, and a decrease in some common geriatric conditions (renal disease and biliary hyperplasia), which may be secondary changes and do not reflect any deleterious effect on a specific organ or the overall health of the animals.

In females at the 10 mg/kg dose level, similar effects were noted, although the severity was less than in the males. There was a transient body-weight depression and the increase in adrenal weight observed at termination was not accompanied by any histological lesion. Plasma and brain cholinesterase were depressed, by not erythrocyte cholinesterase. Serum cholesterol and globulins were mildly depressed and urine specific gravity was elevated. There was also a decreased incidence of biliary hyperplasia.

Depressed body-weight gain and plasma and RBC cholinesterase levels were observed in the 1 mg/kg dose males, while the females of this dose group displayed only a depression in plasma cholinesterase levels.

No increase in tumors of any type was observed in this study.

It is to be noted that the decrease in body weight/body-weight gain observed was less than 10%, although it achieved a statistical level of significance in the 10 mg/kg (both sexes) and 1 mg/kg (males) dose groups. The magnitude of the difference does not appear to be sufficient, by itself, to conclude that the animals were adequately challenged. The changes in serum cholesterol, total protein and globulin, and urine specific gravity may be secondary changes due to the effects of the test material on cholinesterase and the adrenal. Since there was a significant depression in two of the assayed cholinesterase enzyme measurements (brain and plasma) at the 10 mg/kg dose level in both sexes, this dose can be considered adequately high for assessing the oncogenic potential of Chlorpyrifos.

The NOEL for both sexes can be set at 0.1 mg/kg, the LEL at 1 mg/kg, based on decreased plasma and brain cholinesterase activity.

Chlorpyrifos toxicology review

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Pages 12 through 13 are not included in this copy.

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___ Identity of product inert ingredients

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