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DATA EVALUATION RECORD

CHLORPYRIFOS (Dowco 179)

2-Year Chronic Toxicity/Oncogenicity Feeding Study—Rats

CITATION: McCollister, S.B., Kociba, R.J., Gehring, P.J., and Humiston, C.G. Results of two-year dietary feeding studies on Dowco<sup>R</sup> 179 in rats. Unpublished study by Dow Chemical Co. dated September 20, 1971.

REVIEWED BY:

William L. McLellan, Ph.D.  
Project Scientist  
Dynamac Corporation

Signature: Henry Appleton for  
Date: 4/6/84

Henry T. Appleton, Ph.D.  
Program Manager  
Dynamac Corporation

Signature: Henry T. Appleton  
Date: 4/6/84

Cipriano Cueto, Ph.D.  
Department Director  
Dynamac Corporation

Signature: Cipriano Cueto  
Date: 4-6-84

APPROVED BY:

~~JOHN A. QUEST~~  
~~Gary Burin, Ph.D.~~  
EPA Scientist

Signature: John A. Quest  
Date: 4/24/84

DATA EVALUATION RECORD

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STUDY TYPE: 2-year chronic toxicity/oncogenicity feeding study—rats.

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ACCESSION NUMBER: Not available.

MRID NUMBER: 00081270.

LABORATORY: Chemical Biology Research, Dow Chemical USA, Midland Mich.

TEST MATERIAL: Chlorpyrifos [0,0-diethyl-0-(3,5,6-trichloro-2-pyridyl)-phosphorothioate], Dowco<sup>R</sup> 179, was from Lot No. CP523-CD235C and assayed 98.8 percent pure by ultraviolet spectrum and 97.2 percent pure by gas liquid chromatography.

PROTOCOL:

1. Sherman rats, source unspecified, were used in the study. Primary groups of 25/sex/dose level (300 total) were maintained in the study for 2 years and supplementary groups (57/sex/dose level, total 684) were used for interim pathologic examinations and periodic cholinesterase determinations as shown in Table 1. At initiation of dosing the animals were 7 weeks old, and males weighed approximately 165 g and females approximately 135 g. Chlorpyrifos was given in the diet at concentrations which resulted in dosages to the test animals of 0 (control), 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/day. The animals were housed 2/cage in wire mesh cages for 3 months and were individually caged thereafter. Food and water were available ad libitum.
2. An appropriate amount of test compound dissolved in acetone was mixed with ground Purina Lab Chow to prepare a 1 percent premix. Test diets were prepared by mixing an appropriate amount of premix and ground feed. The concentration of chemical in diets was adjusted weekly for the first 3 months and as often as necessary thereafter to maintain the designated dosages (mg/kg/day). Diets were prepared weekly or biweekly. Periodic analysis by a gas liquid chromatography method was used to check the dietary levels of test compound. The test material was stable in feed at room temperature for one week. After 7 weeks storage, recovery was 87-92 percent.

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TABLE 1. Supplementary Groups of Animals

| Purpose                                              | No./group/sex |
|------------------------------------------------------|---------------|
| 1 week ChE <sup>a</sup> determination                | 5             |
| 1 month ChE determination                            | 5             |
| 3 month ChE determination                            | 5             |
| 6 month ChE determination                            | 5             |
| 9 month ChE determination                            | 5             |
| 12 month ChE determination                           | 6             |
| 12 month necropsy                                    | 5             |
| 12 month plus recovery <sup>b</sup> necropsy and ChE | 7             |
| 18 month ChE determination                           | 7             |
| 18 month necropsy                                    | 7             |

<sup>a</sup> ChE, cholinesterase.

<sup>b</sup> Fed dosed diet for 12 months and then control diet for 50 days.

3. Animals were observed "frequently" [sic] for mortality, moribundity and for toxic signs, especially for evidence of cholinergic responses. Body weights were recorded twice weekly for the first month, weekly during months 2-4 and bi-weekly thereafter. Food consumption for the primary groups was recorded weekly for the first 3 months and one week/month thereafter.
4. Hematologic studies were performed on 5 rats/sex of the 0, 1.0 and 3.0 mg/kg groups at 1, 6, 12, 18, and 24 months, except at 12 months 10 females of each of these groups were examined. The parameters measured were hematocrit, hemoglobin, erythrocyte count, and total and differential leukocyte count. Urinalyses were conducted on 5 rats/sex from the above groups at the same time intervals.
5. Blood urea nitrogen (BUN), alkaline phosphatase (AP), and serum glutamic pyruvic transaminase were measured on rats sacrificed at 12 months (5/sex/group), 18 months (7/sex/group), and 24 months (all males and 4-5 females/group).
6. Cholinesterase activity of plasma and erythrocytes was measured on 5-7 rats/sex/ dosage group (supplementary groups) at 1 week, and at 1, 3, 6, 9, 12, and 18 months and on all surviving males and all but 4-5 females/group at the 24 month sacrifice. Brain cholinesterase was measured at 6, 12, and 18 months. In addition, blood and brain cholinesterase activity were measured in a recovery group of 7 rats/sex/dose, maintained on test diets for 12 months and then fed control diets for 7-8 weeks.
7. Animals were sacrificed and necropsies conducted at the following intervals: 12 months, 5 rats/sex/group; 18 months, 7 rats/sex/group; and 24 months, all surviving rats. Brain, heart, liver, kidneys, spleen and testes from each animal were weighed. Portions of the above organs and the following tissues were preserved in formalin: eyes, pituitary, thyroid, parathyroid, trachea, esophagus, lungs, aorta, stomach, pancreas, small intestine, colon, mesenteric lymph nodes, urinary bladder, accessory sex glands, ovaries, uterus, skeletal muscle, sciatic nerve, sternum and bone marrow, adrenals, and any nodule or mass.
8. The preserved tissues were examined microscopically for animals in the 0, 1.0 and 3.0 mg/kg groups at 12 month sacrifice, and for animals in the 0 and 3.0 mg/kg groups at recovery sacrifice, 18 month sacrifice, and those sacrificed at termination of the study. In addition, tissues from all test groups and controls were examined for animals that died during the study.
10. Hematologic data, clinical chemistry data, cholinesterase activity data, final body weight, organ weight and organ/body weight ratio data were analyzed by Student's t-test for significant differences between control and test mean values.

RESULTS:

Observations: It was stated that there were no changes in appearance, signs of toxicity, or cholinergic responses in any animals at any time in the study; however, no data were available to support this.

Mortality: Data on mortality are summarized in Table 2. There was no compound-related effect on mortality or on the times at which deaths occurred. Survival at 18 months ranged from 64 to 92 percent in all groups.

TABLE 2. Mortality Data<sup>a</sup>

| Group                       | Dose (mg/kg/day) |      |      |     |     |     |
|-----------------------------|------------------|------|------|-----|-----|-----|
|                             | 0                | 0.01 | 0.03 | 0.1 | 1.0 | 3.0 |
| <b>Males</b>                |                  |      |      |     |     |     |
| No. of deaths (24 mos.)     | 15               | 16   | 19   | 18  | 12  | 15  |
| 18-month survival (percent) | 68               | 68   | 64   | 80  | 80  | 64  |
| <b>Females</b>              |                  |      |      |     |     |     |
| No. of deaths (24 mos)      | 14               | 11   | 11   | 12  | 8   | 15  |
| 18 month survival (percent) | 80               | 76   | 92   | 92  | 88  | 92  |

<sup>a</sup>In groups of 25 rats/sex started on test.

Body Weights and Food Consumption: The mean body weights of males and females administered test compound were similar to or greater than controls throughout the two-year study. However, mean body weights were presented graphically in the report, means and standard deviations were not tabulated, individual animal data were not present, and statistical analysis was not available. Mean food consumption was similar in all groups of males and females throughout the study.

Hematology: Although there were scattered significant differences in hemoglobin values and white cell counts between control and test groups of rats, there were no time- or dose-related effects and the values were within the normal range. There was no compound-related effect on any other hematologic parameter.

Cholinesterase Activity: Doses up to 0.1 mg/kg/day of chlorpyrifos caused no significant depression of cholinesterase activity of plasma, erythrocyte, or brain when compared to controls. However, there were dose-related depressions of plasma and erythrocyte cholinesterase activity throughout the study in male and female animals at 1.0 and 3.0 mg/kg/day test compound when compared with controls (Tables 3 and 4). The levels of plasma cholinesterase activity were somewhat more depressed in females

TABLE 3. Mean Cholinesterase Activity of Plasma as Percent of Control Value

| Days on Diets                  | Dose Level (mg/kg/day) |       |       |         |       |       |
|--------------------------------|------------------------|-------|-------|---------|-------|-------|
|                                | Males                  |       |       | Females |       |       |
|                                | Control                | 1.0   | 3.0   | Control | 1.0   | 3.0   |
| 7                              | 100                    | 86.7  | 59.0* | 100     | 66.5  | 44.7  |
| 30                             | 100                    | 94.1  | 79.3  | 100     | 55.8* | 33.0* |
| 90                             | 100                    | 97.1  | 70.5* | 100     | 46.6* | 39.3* |
| 180                            | 100                    | 82.4  | 80.2  | 100     | 38.1* | 27.3* |
| 279                            | 100                    | 61.5* | 61.5* | 100     | 31.0* | 26.0* |
| 365                            | 100                    | 80.3  | 66.4* | 100     | 49.5* | 36.1* |
| 365 + 50 recovery <sup>a</sup> | 100                    | 83.4* | 81.8  | 100     | 98.7  | 92.0  |
| 547                            | 100                    | 66.2* | 61.5* | 100     | 47.8* | 34.0* |
| 730                            | 100                    | 73.9* | 67.5* | 100     | 43.7* | 31.8* |

<sup>a</sup>50 days recovery on control diet after 365 days of dosing.

\*Statistically different from control at  $p < 0.05$  when absolute values were compared by the Student t-test.

TABLE 4. Mean Cholinesterase Activity of Erythrocytes as Percent of Control Value

| Days on Diets                  | Dose Level (mg/kg/day) |       |       |         |       |       |
|--------------------------------|------------------------|-------|-------|---------|-------|-------|
|                                | Males                  |       |       | Females |       |       |
|                                | Control                | 1.0   | 3.0   | Control | 1.0   | 3.0   |
| 7                              | 100                    | 86.7  | 40.5* | 100     | 88.2  | 32.1* |
| 30                             | 100                    | 31.1* | 4.9*  | 100     | 34.3* | 4.8*  |
| 90                             | 100                    | 42.2* | 35.9* | 100     | 42.2* | 23.9* |
| 180                            | 100                    | 15.9* | 9.4*  | 100     | 22.0* | 12.2* |
| 279                            | 100                    | 15.8* | 11.3* | 100     | 24.2* | 10.0* |
| 365                            | 100                    | 9.5*  | 1.4*  | 100     | 18.5* | 4.1*  |
| 365 + 50 recovery <sup>a</sup> | 100                    | 91.1  | 105.3 | 100     | 94.6  | 104.6 |
| 547                            | 100                    | 16.0* | 11.5* | 100     | 15.8* | 11.5* |
| 730                            | 100                    | 25.9* | 16.4* | 100     | 32.8* | 18.4* |

<sup>a</sup>50 days recovery on control diet after 365 days of dosing.

\*Statistically different from control at  $p < 0.05$  when absolute values were compared by the Student t-test.

than in males at 1.0 and 3.0 mg/kg/day. When rats were fed test compound in the diets at 1.0 or 3.0 mg/kg/day for one year and then fed control diets for a 7-week recovery period, cholinesterase activity of plasma and erythrocytes returned to normal.

Brain cholinesterase activity was depressed at all sampling times in both males and females at 3.0 mg/kg/day and was marginally depressed at 1.0 mg/kg/day when compared to controls. Animals fed the test compound for a year at 3.0 mg/kg/day and then control diets for a 7-week period were considered to have brain normal cholinesterase activity (Table 5) on the bases of the expected biological variation of cholinesterase activity and the variation of the methodology used to determine this activity.

TABLE 5. Mean Cholinesterase Activity of Brain as Percent of Control Value

| Days on<br>Diets                  | Dose Level (mg/kg/day) |       |       |         |       |       |
|-----------------------------------|------------------------|-------|-------|---------|-------|-------|
|                                   | Males                  |       |       | Females |       |       |
|                                   | Control                | 1.0   | 3.0   | Control | 1.0   | 3.0   |
| 180                               | 100                    | 97.3  | 46.9* | 100     | 90.7* | 61.4* |
| 365                               | 100                    | 88.0* | 45.2* | 100     | 92.6  | 47.4* |
| 365 + 50<br>recovery <sup>a</sup> | 100                    | 98.0  | 92.9* | 100     | 98.2  | 90.3  |
| 547                               | 100                    | 90.3* | 70.5* | 100     | 92.1* | 62.6* |
| 730                               | 100                    | 84.2* | 60.4* | 100     | 95.1  | 57.5* |

<sup>a</sup>50 days recovery on control diet after 365 days of dosing.

\* Statistically different from control at  $p < 0.05$  when absolute values were compared by the Student t-test.

Other Clinical Chemistry: There were no compound-related changes in blood urea nitrogen, alkaline phosphatase, or serum glutamic pyruvic transaminase.

Urinalysis: There were no compound-related effects on the urinary parameters determined.

Organ Weights: At the one year sacrifice there were sporadic increases in heart, liver, and kidney weights and organ/body weight ratios in dosed females compared to controls, but the increases were not dose-related nor were they considered toxicologically significant. At the eighteen month sacrifice, organ weights were similar in treated and control males and females. At the two-year sacrifice there were slight but significant increases in the weights of kidney, liver, and spleen in males at the 3.0 mg/kg/day dose compared to controls, but the organ to body weight ratios were not significantly increased when compared to controls.

Gross Necropsy: Some gross lesions were noted, but there was no increased incidence of any lesion in any dosed group of males or females when compared with controls. There was a tabulation of gross alterations for individual animals that died during the study. For animals sacrificed at termination of the study, individual gross findings were not presented, but summary tables of lesions indicated that tissues from all animals were grossly examined. Summary tables of gross lesions (but no individual data) were also present for animals at interim sacrifices.

Histopathology: For animals that died or were sacrificed moribund during the study, there was a tabulation of histopathologic alterations for individual animals. For animals at interim sacrifices and those sacrificed at study termination, individual histopathologic data were not presented.

A summary of incidence of non-neoplastic lesions for animals at interim sacrifices is presented in Table 6. Summary data was present for control and high dose animals; in addition, summary data was present for the 1.0 mg/kg/day group at the 12 month sacrifice. No compound-related effects were noted.

Table 7 summarizes non-neoplastic lesions in animals in the control and 3.0 mg/kg/day groups that died or were sacrificed at study termination. This tabulation utilized individual data for animals that died and summary data for animals that were sacrificed. There was no increased incidence in lesions in the 3.0 mg/kg/day group of males or females when compared to controls. Table 8 summarizes the number of animals in the control and 3.0 mg/kg/day groups with neoplastic lesions. Individual data were only available for animals that died. There was no increased incidence of any tumor in males or females at 3.0 mg/kg/day when compared with controls. The report listed the tumors in animals in lower dose groups but this data could not be validated because it was based on summary data and only tissues with grossly observed masses were examined histologically.

#### DISCUSSION:

Insufficient numbers of animals were employed to satisfy core guideline data for oncogenicity testing but adequate numbers were used to satisfy core minimum data for oncogenicity. Adequate numbers of animals were utilized for chronic toxicity testing; greater than 50 percent survived 18 months and greater than 25 percent survived 24 months.

The histopathology data was limited, since data for individual animals was only available for animals that died or were sacrificed moribund; only summary data was present for animals sacrificed at termination of the study.

The study was further limited since there were no available data on clinical observations or individual body weight data. Hematology data was limited since only at 12 months were 10 animals/sex in the control and high dose groups examined; at other intervals, 5 animals/sex/dose were examined. Urinalyses were performed at the usual intervals in the control, 1.0 and 3.0 mg/kg/day groups but only 5 animals/sex/dose were

TABLE 6. Incidence of Non-Neoplastic Lesions at Interim Sacrifices

| Organ/Lesion                      | Group (mg/kg/day) |     |     |         |     |     |                    |     |     |         |     |     |                   |     |     |         |  |  |
|-----------------------------------|-------------------|-----|-----|---------|-----|-----|--------------------|-----|-----|---------|-----|-----|-------------------|-----|-----|---------|--|--|
|                                   | 12 Mos. Sacrifice |     |     |         |     |     | Recovery Sacrifice |     |     |         |     |     | 18 Mos. Sacrifice |     |     |         |  |  |
|                                   | Males             |     |     | Females |     |     | Males              |     |     | Females |     |     | Males             |     |     | Females |  |  |
|                                   | 0                 | 1.0 | 3.0 | 0       | 1.0 | 3.0 | 0                  | 3.0 | 0   | 3.0     | 0   | 3.0 | 0                 | 3.0 | 0   | 3.0     |  |  |
| Lungs,<br>chronic pneumonia       | 4/4               | 5/5 | 4/5 | 5/5     | 4/5 | 5/5 | 4/7                | 5/6 | 4/7 | 6/6     | 6/6 | 5/5 | 4/7               | 6/6 | 4/7 | 6/6     |  |  |
| minimal                           | -                 | -   | -   | -       | -   | -   | 2/7                | 1/6 | 3/7 | -       | -   | -   | 2/7               | -   | -   | -       |  |  |
| moderate to severe                | 1/4               | 3/5 | -   | 4/5     | 4/4 | 5/5 | -                  | -   | -   | -       | -   | -   | -                 | -   | -   | -       |  |  |
| submucosal tracheitis             | -                 | -   | -   | -       | -   | -   | -                  | -   | -   | -       | -   | -   | -                 | -   | -   | -       |  |  |
| Liver,<br>mononuclear cell foci   | 1/4               | 1/5 | -   | 3/5     | 3/4 | 5/5 | 3/7                | 6/6 | 4/7 | 4/6     | -   | -   | 1/7               | -   | -   | -       |  |  |
| Kidney,<br>glomerular alterations | 2/4               | 3/5 | 4/5 | 4/5     | 3/4 | 5/5 | -                  | -   | -   | -       | -   | -   | -                 | -   | -   | -       |  |  |
| cast formation                    | 2/4               | 3/5 | 4/5 | 4/5     | 3/4 | 4/5 | 2/7                | 4/6 | 5/7 | 5/6     | 4/6 | 3/5 | 3/7               | 4/6 | 3/7 | 4/6     |  |  |
| minimal chron. nephritis          | -                 | -   | -   | -       | -   | -   | -                  | -   | -   | -       | 1/6 | 2/5 | 3/7               | 1/6 | -   | -       |  |  |
| mod. to severe nephritis          | -                 | -   | -   | -       | -   | -   | 5/7                | -   | -   | -       | -   | -   | -                 | -   | -   | -       |  |  |
| dilation of renal<br>tubules      | 3/4               | 2/5 | 4/5 | 2/5     | 1/4 | 2/5 | -                  | -   | -   | -       | -   | -   | -                 | -   | -   | -       |  |  |

The data are expressed as: number of occurrences/number of animals examined histologically.

TABLE 7. Number of Animals with Non-Neoplastic Histopathologic Lesions

| Organ/Lesion               | Dose Level (mg/kg/day) |     |         |     |
|----------------------------|------------------------|-----|---------|-----|
|                            | Males                  |     | Females |     |
|                            | 0                      | 3.0 | 0       | 3.0 |
| No. of animals examined    | 24                     | 25  | 25      | 25  |
| <u>Lung</u>                |                        |     |         |     |
| chronic murine pneumonia   | 22                     | 18  | 24      | 16  |
| <u>Liver</u>               |                        |     |         |     |
| mononuclear cell foci      | 4                      | 1   | 7       | 2   |
| pleocellular foci          | -                      | -   | 2       | 1   |
| <u>Kidney</u>              |                        |     |         |     |
| dilation of renal pelvis   | -                      | 1   | -       | -   |
| chronic nephritis:         |                        |     |         |     |
| -severe                    | 2                      | 3   | 5       | 3   |
| -minimum to moderate       | 14                     | 9   | 14      | 13  |
| <u>Heart</u>               |                        |     |         |     |
| myocardial degeneration    | 11                     | 9   | 8       | 12  |
| mesenteric periarteritis   | -                      | 2   | -       | 3   |
| <u>Adrenal</u>             |                        |     |         |     |
| hematocyst                 | 1                      | 2   | 7       | 7   |
| <u>Testes</u>              |                        |     |         |     |
| atrophy                    | 1                      | 2   |         |     |
| <u>Stomach</u>             |                        |     |         |     |
| focal gastritis            | -                      | 1   | -       | 1   |
| <u>Spleen</u>              |                        |     |         |     |
| hyperplasia                | 1                      | -   | 1       | 2   |
| cellular depletion         | 1                      | -   | -       | -   |
| extramedullary hemopoiesis | 2                      | 2   | 2       | 2   |

<sup>a</sup>Prepared by this reviewer using individual histopathology data for animals that died during the study and summary incidence data for animals sacrificed at 24 months.

TABLE 8. Number of Animals with Neoplastic Histopathologic Lesions

| Organ/Lesion               | Dose Level (mg/kg/day) |     |         |     |
|----------------------------|------------------------|-----|---------|-----|
|                            | Males                  |     | Females |     |
|                            | 0                      | 3.0 | 0       | 3.0 |
| Malignant lymphoma         | 2                      | 0   | 0       | 1   |
| Skin, fibrosarcoma         | 1                      | 0   | 0       | 0   |
| Skin, subcutaneous sarcoma | 0                      | 1   | 0       | 0   |
| Thyroid, carcinoma         | 0                      | 1   | 2       | 0   |
| Thyroid adenoma            | 0                      | 0   | 1       | 0   |
| Adrenal, pheochromocytoma  | 1                      | 1   | 0       | 0   |
| Pituitary, adenoma         | 0                      | 0   | 5       | 6   |
| Pancreas, adenoma          | 0                      | 0   | 1       | 0   |
| Uterus, adenoma            | -                      | -   | 1       | 0   |
| Uterus, polyps             | -                      | -   | 2       | 0   |
| Uterus, fibropapilloma     | -                      | -   | 1       | 0   |
| Mammary, fibroadenoma      | -                      | -   | 2       | 2   |
| No. of animals examined    | 24                     | 25  | 25      | 25  |

<sup>a</sup>Prepared by this reviewer using individual histopathology data for animals that died during the study and summary incidence data for animals sacrificed at 24 months.

studied. Clinical chemistry determinations were made on 10 animals group only for males at 24 months. For males at other intervals and for females at all intervals less than 10 animals/group had blood chemistry determinations.

Although there were no cholinergic signs noted even at the highest dose, there was severe inhibition of erythrocytes cholinesterase (96-98 percent) at the one year interval in animals at 3.0 mg/kg/day, and approximately a 50 percent inhibition of brain cholinesterase at this level and interval when compared with controls. This would indicate that a maximum tolerated dose was probably used. However, no other compound-related toxicity was noted.

Since only summary data for gross findings and histopathology were present for interim sacrificed and study termination sacrificed animals, the study as reported is considered as seriously limited. If the registrant can supply data on clinical observations, individual animal data on body weights, and individual animal gross and histopathologic observations on all required tissues, the core classification may be reconsidered.

#### CONCLUSIONS:

Chlorpyrifos was not oncogenic to male or female Sherman rats when fed at levels of up to and including 3.0 mg/kg/day for two years. However, individual histopathology data were not present to support this conclusion. The only compound related effects were depression of cholinesterase activity of plasma and erythrocytes at dose levels of 1.0 and 3.0 mg/kg/day and of brain at dose levels of 3.0 mg/kg/day. Inhibition of cholinesterase activity was reversible; rats maintained on diets free of chlorpyrifos for 7 weeks after a year of dosing showed recovery of cholinesterase to control levels. Based on inhibition of cholinesterase activity, a LEL of 1.0 mg/kg/day and a NOEL of 0.1 mg/kg/day can be tentatively established.

CORE CLASSIFICATION: Supplementary.