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DATA EVALUATION RECORD

TRICHLORFON

Chronic Oral Toxicity

CITATION: Doull J, Vesselinovitch D, Root M, Cowan J, Meskauskas J, Fitch F. 1962. Chronic oral toxicity of Dylox to male and female rats. (An unpublished study submitted by Chemagro Division, Mobay Chemical Corp., Report No. 19246, May 31, 1962.)

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7 pages

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STUDY TYPE: Chronic oral toxicity in rats.

CITATION: Doull J, Vesselinovitch D, Root M, Cowan J, Meskauskas J, Fitch F. 1962. Chronic oral toxicity of Dylox to male and female rats. (An unpublished study submitted by Chemagro Division, Mobay Chemical Corp., Report No. 19246, May 31, 1962.)

ACCESSION NUMBER: 090786.

MRID NUMBER: Not available.

LABORATORY: Departments of Pharmacology and Pathology, University of Chicago, Chicago, Illinois.

TEST MATERIAL: The test material was identified as Dylox technical, a trade name for trichlorfon (O,O-dimethyl-2,2,2-trichloro-1-hydroxyethyl-phosphonate). A single sample (No. 888692/169, Lot 20886) was provided by the sponsor and was used throughout the study. No information was provided on the stability of the test material nor were the test diets analyzed for Dylox content.

PROCEDURES:

1. A total of 300 Sprague-Dawley rats (150 of each sex) were obtained from an unspecified source; at the start of the feeding period they were 4 weeks old and weighed 65-88 g. The animals were divided into 6 groups (25 rats/sex/group), were housed individually in temperature-controlled quarters (78 ± 5°F), and were provided a test diet and water ad libitum. Test diets were prepared weekly by mixing an appropriate amount of Dylox with ground food (Rockland Rat Diet) to obtain dietary levels of 0, 50, 250, 500, and 1000 ppm. Two groups were fed the control diet (0 ppm Dylox).

2. Each animal was weighed weekly during the first 3 months of the study and biweekly thereafter. Food consumption was measured twice weekly for the first 4 weeks, and the rats were examined for signs of toxicity twice weekly throughout the study.

3. After 2 years of feeding, 5 female rats from each group were sacrificed and the cholinesterase activity of brain, submaxillary glands, serum, and erythrocytes determined by the method of DuBois and Mangun (Proc. Soc. Exp. Biol. Med. 64:134, 1947). Similar determinations were

performed on 5 male rats from each group; however, the mortality rate among the male animals necessitated sacrifice at 17 months to achieve the desired number of animals.

4. Concurrent with the cholinesterase determinations (2 years for the females and 17 months for the males), the sacrificed animals were examined grossly, and brain, liver, kidney, spleen, heart, lung, and testis (for males) were removed and weighed. Samples of 18 tissues from each of these animals were prepared and examined for histopathologic changes. In addition, animals that died during the feeding period were necropsied and their tissues prepared for histopathologic examination except when autolysis was advanced.

RESULTS:

Food Consumption/Body Weight: No differences were observed in the daily food consumption of the Dylox-treated groups compared to the control groups during the first 30 days of the study. The authors stated that infrequent measurements made during the remainder of the study did not reveal any differences. No differences were evident in the body weight gain of the female rats of the dosed groups compared to control throughout the study. Male rats fed diets containing 500-ppm Dylox or less showed no differences in body weight gain relative to the concurrent control animals. At the 1000-ppm level, weight gain was distinctly less than the control at 4 months; average body weights for this group remained 10-20 percent below the control group averages for the remainder of the study.

Survival/Clinical Observations: An effect on survival was noted at the 1000-ppm dose level. The median survival time for animals of both sexes in this group was reported to be about 15 percent less than the median survival times for the other groups. Specific times were not reported; the following median survival times were estimated from the graphs of survival presented in the report: females at 1000 ppm- 16 months, females of other groups- 18 months; males at 1000 ppm- 12 months, males of other groups- 14 months. No statement was made on the observation of the animals for signs of toxicity.

Cholinesterase Activity: Serum cholinesterase activity was substantially affected (i.e., a depression of more than 25 percent below control) in animals of both sexes in the 500- and 1000-ppm groups (Table 1). Cholinesterase activity in erythrocytes and submaxillary glands was depressed at 1000 ppm, but not at the other dose levels (Table 1); brain cholinesterase activity was not affected at any level.

Organ Weights/Gross Pathological Examination: No dose-related differences were observed in either the organ weights or the organ-to-body weight ratios. A check of the organ-to-brain weight ratios performed by the reviewer revealed no dose-related differences. Gross examination at sacrifice revealed that 6 of the Dylox-fed animals had mammary gland tumors (1 at 250-ppm, 3 at 500-ppm, and 2 at 1000-ppm); no tumors of this type were found in the control animals. Other lesions were observed

TABLE 1. Effect of Dietary Dylox on Cholinesterase Activity

Dose Level (ppm)	Sex	Cholinesterase Activity[a]		
		Serum	Erythrocyte	Submaxillary Gland
500	M	76	—[b]	--
	F	73	—	—
1000	M	60	70	74
	F	63	85	84

[a]Percent of control.
[b]Difference from control was not greater than 15 percent.

sporatically distributed among both control and dosed groups (e.g., consolidation and abscess formation in the lungs).

Histopathologic Examination: Several dose-related histopathologic changes were noted in the animals (5/sex/group) examined at the termination of the study. Mammary gland tumors, described as fibroadenomas, adenocarcinomas, and undifferentiated sarcomas, were found with greater incidence in the animals of the Dylox-fed groups than in the controls (Table 2). In addition, 2 females animals of the 1000-ppm group had tubular androblastomas of the ovary (a nonmalignant tumor); no tumors of this type were found in the control animals. Other findings (focal inhibition of spermatogenesis in males, the absence of primary follicules and ova in females, and arteritis) were also noted exclusively in the animals fed Dylox (Table 2). Other histopathologic changes were found with similar incidences in control and dosed groups. Observations on individual animals were not reported, and no histopathologic data were provided for animals that died during the study.

DISCUSSION:

This study contained several deficiencies that limit its use in the assessment of the chronic toxicity of Dylox. The primary deficiency in this study was the inadequate examination of animals for histopathologic changes. Although the procedures stated that tissues from animals dying during the study were preserved for examination (and the mortality rate was high), no results from the examination of these animals were presented nor was any statement made on their disposition.

In addition, and in accordance with the protocol, only 5 surviving animals of each sex and group were examined at the study's termination. A second deficiency was that the mortality rate among the male animals was

TABLE 2. Histopathologic Findings at Sacrifice in Rats Fed Dylox

Organ/Finding	Incidence[a] 0 ppm M	0 ppm F	50 ppm M	50 ppm F	250 ppm M	250 ppm F	500 ppm M	500 ppm F	1000 ppm M	1000 ppm F
Mammary gland- tumor	0	0	0	0	0	1[b]	0	3[c]	0	3[d]
Testis- focal inhibition of spermatogenesis	0	-	0	-	0	-	0	-	3	-
Ovary- absence of primary follicles and primative ova	-	0	-	0	-	1	-	2	-	5
-tubular androblastoma	-	0	-	0	-	0	-	0	-	2
Blood vessels- necrotizing arteritis	0	0	2	1	0	1	3	2	2	0

[a] 5 animals/sex/group were examined.

[b] A fibroadenoma.

[c] A fibroadenoma and 2 adenocarcinomas.

[d] An adenocarcinoma (acinar type), a fibroma, and a "poorly differentiated malignant - appearing tumor resembling a sarcoma."

such that an adequate number of animals were not exposed to the test material over a substantial fraction of their expected lifetimes. Fifty percent mortality occurred at approximately 12-14 months for the males; no discussion of this high mortality was provided by the authors. The third major deficiency was that no clinical pathology studies (hematology, clinical chemistry, and urinalysis) were performed with the exception of the determination of cholinesterase activity at the termination of the study. Two other points were noted: none of the results of the biweekly clinical observations were presented and, although the use of a second control group was specified in the procedures, no information on this group was presented in the remainder of the report.

Although the incidences of several histopathologic findings were higher in the dosed groups compared to the controls, the small number of animals that were examined precluded meaningful interpretation of these data. For example, the most notable finding was the higher incidence of mammary tumors in the dosed animals. However, this tumor has a high spontaneous incidence in this strain of rats, and therefore the reported zero incidence in the 5 controls may not be representative of the historical rate or of the total control group. In their discussion, the authors considered the problem of determining the actual number of animals with tumors given the limited number of animals examined and resorted to estimating the incidence of mammary tumors by counting the number of "grossly detectable mammary tumors." They stated in their discussion that the incidence of these tumors was higher in the high-dose animals versus the controls (25 versus 14 percent, respectively) and the tumors appeared earlier (first tumors at 1.1 versus 1.7 years, respectively); however, the authors did not state how these data were determined. Further clarification is needed of the authors' data on mammary tumors for this finding to be properly interpreted. Other histopathologic lesions reported to have higher incidences in the dosed animals than in controls may also be related to treatment; however, due to the inadequate histopathologic examinations, these results are suspect. This study therefore is useful in that it identifies potential adverse effects, primarily the possible induction of tumors; a more thorough study with adequate histopathology is needed.

CONCLUSIONS:

Sprague-Dawley rats that were maintained on diets containing Dylox (trichlorfon) for 17 months (males) or 24 months (females) showed depression of cholinesterase activity in serum (at 500- and 1000-ppm dietary Dylox), erythrocytes (1000-ppm), and submaxillary gland (1000-ppm). No effect on cholinesterase activity was seen at the lower doses tested (50, 100, and 250 ppm). Animals in the 1000-ppm group had lower body weight gain (males only) and higher mortality (both sexes) when compared to the control group. Histopathologic examination of only 5 animals/sex/group at the study's termination suggested that at 1000-ppm Dylox, spermatogenesis was inhibited in males and maturation of primary ovarian follicules was suppressed in females. The study also reported a possible induction of mammary gland tumors by the test material. These

findings are indicative of the need for a chronic toxicity and/or oncogenicity study with a more thorough protocol, particularly involving histopathology.

CORE CLASSIFICATION: Supplementary.

This core classification is based on the following deficiencies: 1) insufficient numbers of animals were examined for histopathology, and 2) no clinical pathology studies were conducted.