4-8-86

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April 8, 1986

DATA EVALUATION RECORD

OXYCHLORDANE

Subchronic Oral Toxicity in Rats

STUDY IDENTIFICATION: Laveglia, J., Naas, D. J., and Tasker, E. J. 90-Day dietary study in rats with oxychlordane. (An unpublished study No. WIL15133 prepared by WIL Research Laboratories, Inc., Ashland, OH, for Velsicol Chemical Corp., Chicago, IL.; dated February 22, 1985.) Accession No. 257803.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I Cecil Felkner

Date: 4-8-86

1. CHEMICAL: Oxychlordane technical.

2. TEST MATERIAL: The test material, oxychlordane technical, Anal. Ref. No. RA 20730, 99.82 percent pure, was described as a white crystalline material.

3. STUDY/ACTION TYPE: Subchronic toxicity study in rats.

4. STUDY IDENTIFICATION: Laveglia, J., Naas, D. J., and Tasker, E. J. 90-Day dietary study in rats with oxychlordane. (An unpublished study No. WIL15133 prepared by WIL Research Laboratories, Inc., Ashland, OH, for Velsicol Chemical Corp., Chicago, IL.; dated February 22, 1985.) Accession No. 257803.

5. REVIEWED BY:

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7. CONCLUSIONS:

A. Oxychlordane at dietary levels of 50 ppm in male rats and 25 or 50 ppm in female rats caused hyperactivity, convulsions, and deaths. There were significant ($p < 0.01$) decreases in mean body weights accompanied by decreases in food consumption in males and females receiving 50 ppm. At terminal sacrifice, there were increases in mean liver weights and mean weights of liver relative to body weight or brain weight in males receiving 25 or 50 ppm and females receiving 25 ppm. Microscopically, there was a significant ($p \leq 0.05$) increase in hepatocyte megalocytosis in males receiving 5, 25, or 50 ppm and in females receiving 25 or 50 ppm. Spongiosis of the white matter of the brain and spinal cord was also significantly increased in males and females receiving 25 and 50 ppm oxychlordane when compared to controls. Significant increases in serum cholesterol levels in males receiving 25 or 50 ppm and females receiving 50 ppm correlated with changes in liver weights and histologic lesions in the liver. Histologic changes in the central nervous system could be related to the occurrence of convulsions. A NOEL could not be established because there were significant but minimum effects on the liver of males receiving 5 ppm. The LOEL was 5 ppm, the lowest dose tested.

B. The study is Core Minimum; a LOEL could not be definitively determined.

8. RECOMMENDATIONS:

The study should be repeated using lower dose levels because the highest dose tested caused excessive mortality and a LOEL could not be established from the lowest dose tested.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (Detailed materials and methods are presented in Appendix A; the following is a summary.)

1. Charles River CD rats (Charles River Breeding Laboratories, Inc.) were used in the study. Rats (26 days old) were acclimated to laboratory conditions for 19 days and then caged individually in a temperature- and humidity-controlled room with a 12-hour light/dark cycle. Food (Purina Certified Rodent Chow No. 5002) and water were offered ad libitum. At

[1]Only items appropriate to the DER are included.

initiation, mean body weights of male groups were between 156-157 g and female groups were between 125-126 g. Groups of 20 males and 20 females were fed diets containing 0, 5, 25, or 50 ppm oxychlordane (during the first week the high-dose group received 75 ppm). Diets were analyzed for homogeneity prior to study initiation; diets were prepared weekly, and samples were analyzed for test material concentration at 1, 2, 3, 4, 8, and 12 weeks.

2. All rats were observed twice daily for mortality and toxic signs, and they received detailed examinations weekly. Body weights and food consumption were recorded weekly. Ophthalmologic examinations were conducted at the pretest and 12 week intervals.

3. Clinical laboratory studies were conducted on 10 randomly selected animals per sex prior to study initiation and on 10 animals/sex/group at 6 and 12 weeks. Animals were fasted overnight prior to collecting blood and urine. Nine hematologic parameters, 19 clinical chemistry parameters, and 11 urinary parameters were measured.

4. Complete gross examinations were conducted on all animals that died, were sacrificed moribund, or were killed at a scheduled sacrifice. Organ weights were obtained from all animals at scheduled sacrifices; these included brain, heart, liver, kidneys, ovaries, and testes. Samples of plasma and blood cells (2 mL), renal fat (1 g), and liver (5 g) were collected, frozen, and sent to the sponsor for analysis of residues. Approximately 33 tissues from the animals were fixed in formalin. Microscopic examination was conducted on a complete set of tissues for control and high-dose groups of animals and for kidney, liver, lung, gross lesions, brain, and spinal cord of all animals in the low- and mid-dose groups.

5. Continuous data were analyzed by one-way analysis of variance and pair-wise comparison of control and dose groups evaluated with the Dunnett's test.

B. Protocol: Materials and Methods are presented in lieu of a protocol (see Appendix A).

12. REPORTED RESULTS:

Dietary Analysis: Test material was stable in the diets. When diets were stored for 18 days at ambient temperature, oxychlordane recovery was 94 to 95 percent. The test material was homogeneous in the diets. Analysis of diets conducted at seven intervals throughout the study indicated that dietary levels of oxychlordane were 94.4 to 96.3 percent of theoretical values.

Mortality and Clinical Observations: There was an increase in mortalities of females receiving 25 and 50 ppm oxychlordane. Six high-dose females died on day 7; by day 14, 12 animals had died and 2 more died by day 19. The remaining six females in the high-dose group were sacrificed on day 19. Survival was lower for mid-dose females than for controls, with 65 percent surviving to termination; however, there were no deaths in control or low-dose females (Table 1). Survival was also lower in high-dose males (35 percent) than in controls (100 percent). Convulsions were noted in four high-dose rats (two males and two females) and two mid-dose females. Hyperactivity was noted in 15 high-dose animals (10 males and 5 females) and in 1 mid-dose female (Table 2).

There were no compound-related effects on ophthalmologic findings at study termination.

Body Weights and Food Consumption: There were compound-related decreases in mean body weights in high-dose males and females noted as early as week one and continuing throughout the study (Table 3). Body weights in both males and females receiving 5 or 25 ppm were similar to controls. Food consumption (expressed as g/kg body weight/day) was significantly ($p \leq 0.01$) decreased in high-dose males and females during week 2 (Table 4), but was similar to control values in low- and mid-dose males and females. There was a slight increase in males later in the study, the increase being significantly ($p < 0.05$) greater than controls during weeks 8, 9, 11, and 12. Food consumption (expressed as g/animal/day) was significantly ($p < 0.05$) decreased compared to controls for high-dose males during weeks 2-6, high-dose females during weeks 2 and 3, and mid-dose females during week 2.

Hematology and Urinalysis: There were no compound-related effects involving hematologic or urinary parameters. All values were considered within the normal range. The only significant change noted ($p \leq 0.05$) was a decrease in mean corpuscular hemoglobin concentration at 12 weeks in males receiving 50 ppm oxychlordane.

Clinical Chemistry: There was a significant increase in mean cholesterol levels in high-dose males and mid-dose females at weeks 7 and 13 ($p \leq 0.01$). There was also an increase in mid-dose males at 7 weeks (not significant, $p > 0.05$) and 13 weeks (significant, $p < 0.05$) (Table 5). A significant decrease ($P \leq 0.05$) was noted in lactic dehydrogenase values for mid- and high-dose males at 13 weeks and a decrease was also noted (not significant, $p > 0.05$) in high-dose males at 7 weeks (Table 6). Other significant changes occurred, but there were no dose- or time-consistent patterns and the changes were not considered of toxicologic importance. Increases in calcium and phosphorous in high-dose males at weeks 7 and 13 were considered not of toxicologic importance because the values were within the normal range.

TABLE 1. Cumulative Mortality and Percent Survival in Rats Fed Oxychlordane for 13 Weeks[a]

Dietary Level (ppm)	Mortalities (and percent survival) at the end of week		
	3	8	13[d]
Males			
0	0 (100)[b]	0 (100)	0 (100)
5	0 (100)	0 (100)	1 (95)
25	0 (100)	0 (100)	0 (100)
50[a]	2 (90)	9 (55)	13 (35)
Females			
0	0 (100)	0 (100)	0 (100)
5	0 (100)	0 (100)	0 (100)
25	0 (100)	4 (80)	7 (65)
50[c]	14 (30)	- [d]	- [d]

[a] The terminal sacrifices were between days 91-97.

[b] The values in parentheses are percent survival.

[c] The dietary level for the first 6 days of the study was 75 ppm.

[d] Six females surviving were sacrificed on day 19.

TABLE 2. Number of Animals with Clinical Observations After Feeding of Oxychlordane

	Dietary Level (ppm)			
	0	5	25	50
Males				
Convulsions	0	0	0	2[a]
Abnormal gait	0	1	0	0
Hyperactivity	0	0	0	10[b]
Females				
Convulsions	0	0	2[c]	2[d]
Hyperactivity	0	0	1[e]	5

[a] Days 0-30, one rat; days 31-60, two rats.

[b] Days 0-30 only.

[c] Days 31-60.

[d] Days 0-60.

[e] Days 31-60.

TABLE 3: Selected Mean Body Weight of Rats Fed Oxychlordane for 13 Weeks

Week	Mean Body Weight (g) at Dietary Level (ppm)		
	0	25	50
		Males	
1	217.5±13.3[a] (20)[b]	214.2±24.8 (20)	213.4±19.6 (20)
3	312.7±16.5 (20)	309.3±25.7 (20)	278.3±19.3** (18)
8	437.6±34.8 (20)	432.0±38.1 (20)	380.0±30.5** (11)
14	501.9±45.0 (20)	500.9±45.2 (20)	450.6±26.0** (7)
		Females	
1	162.6±16.1 (20)	159.8±10.3 (20)	160.2±11.8 (20)
3	213.1±23.7 (20)	201.8±14.3 (20)	188.4±12.8* (8)
8	277.0±33.7 (20)	268.5±26.5 (16)	- 0
14	302.0±42.2 (20)	295.4±26.0 (13)	- 0

[a]Mean ± standard deviation.

[b]Number in parenthesis is the sample size.

*Statistically different than control value ($p < 0.05$).

**Statistically different than control value ($p < 0.01$).

TABLE 4. Mean Food Consumption in Rats Fed Oxychlordane for 13 Weeks

Week	Mean Food Consumption (g/kg/day) at Dietary Level (ppm)			
	0	5	25	50
			Males	
1	123.9 ±4.90[a] (20)[b]	124.5 ±4.73 (20)	122.4 ±18.98 (20)	122.8 ±15.54 (20)
2	102.8 ±4.51 (20)	105.1 ±4.94 (20)	103.8 ±4.86 (20)	83.9* ±12.01 (20)
3	90.6 ±4.42 (20)	91.5 ±4.73 (20)	93.9 ±6.97 (20)	91.5 ±7.12 (18)
			Females	
1	123.7 ±4.34 (20)	129.1 ±24.41 (20)	124.5 ±6.06 (20)	124.8 ±6.86 (20)
2	118.1 ±17.05 (20)	109.1 ±7.79 (20)	106.6 ±23.19 (20)	79.8* ±10.75 (12)
3	103.9 ±8.44 (20)	100.3 ±4.52 (20)	106.9 ±15.89 (20)	98.6 ±11.21 (8)

[a]Mean ± standard deviation.

[b]Number in parenthesis is the sample size.

*Statistically different from control value ($p \leq 0.01$).

TABLE 5. Mean Cholesterol Levels (mg/dL) in Rats Fed Oxychlordane for 13 Weeks

Dietary Level (ppm)	Males		Females	
	7 Weeks	13 Weeks	7 Weeks	13 Weeks
0	53.5±11.5[a]	72.6±17.3	62.1± 9.4	75.1±14.2
5	54.5±11.7	73.0±14.8	70.2±11.3	91.8±19.3
25	74.5±23.1	97.3±25.5*	87.2±18.0**	114.2±28.1**
50	93.2±26.1**	111.3±21.7**	-	-

[a]Standard deviation.

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

**Significantly different from control value ($p \leq 0.01$).

TABLE 6. Mean Lactic Dehydrogenase Levels (U/L) in Male Rats Fed Oxychlordane for 13 Weeks

Dietary Level (ppm)	Enzyme Level (U/L) at Week 7	13
0	1403±534	1910±828
5	1650±551	1684±608
25	1436±572	1169±501*
50	1035±553	1195±385*

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

Organ Weights: There were significant ($p \leq 0.01$) increases in mean absolute liver weights and liver weight relative to body weight or brain weight in males receiving 25 or 50 ppm and in females receiving 25 ppm. There were apparent dose-related trends (Table 7). High-dose males also had significant increases in brain ($p \leq 0.05$), kidney ($p \leq 0.01$), and heart ($p \leq 0.05$) weights relative to body weight, but there were no significant increases in the corresponding absolute organ weights.

Gross Pathology: There were no compound-related macroscopic findings in animals sacrificed at study termination (between days 91-97). In animals that died during the study, there was an increase in hemorrhagic areas in brain, intestine, and thymus and increased congestion of kidneys, liver, and lungs, mainly in high-dose animals (Table 8).

Histopathology: Table 9 summarizes histopathologic findings. Compound-related microscopic lesions were found in the liver and central nervous system. There was an increase in megalocytosis of centrilobular and midzonal parenchymal cells in males receiving 5, 25, and 50 ppm and females receiving 25 and 50 ppm. Changes in the livers of low-dose males were all minimal or mild. Severity of the lesion as well as incidence was dose-related in males (Table 9); severity was also related to the duration of dosing. For example, in high-dose males that survived to final sacrifice, all seven had moderate to severe megalocytosis. On the other hand, for males that died, 7/13 had moderate to severe lesions and 5/13 had minimal to mild lesions. The incidence of megalocytosis of the liver was significantly increased in females receiving 25 and 50 ppm. The lower incidence and lesser severity in high-dose females (50 ppm group) when compared to high-dose males were probably related to early deaths and early termination in females; all six females receiving 50 ppm that were sacrificed at day 19 had mild megalocytosis. Two of 14 high-dose females that died had moderate lesions and 6 of 14 had minimal to mild lesions. For both sexes in several animals, the increased megalocytosis was accompanied by vacuolization of centrilobular cells of the liver. The report noted that in males receiving 25 and 50 ppm the megalocytosis in the liver was correlated with increased liver weights and serum cholesterol but the correlation in females was not as strong because of early deaths in the 25 and 50 ppm dosed groups.

There was an increased incidence in spongiosis in the central nervous system in both males and females receiving 25 and 50 ppm (Table 9). These lesions were characterized by microcystic vacuolization of areas of white matter in the brain and less frequently in the spinal cord. In males, most of these lesions were graded minimum to mild; they were more severe in high-dose females that were sacrificed at 19 days. No other histologic lesions were considered compound related. An increase in chronic progressive nephropathy was noted in males at final sacrifice.

TABLE 7. Absolute and Relative Liver Weights at Terminal Sacrifice in Rats Fed Oxychlordane for 90 Days

	Dietary Level (ppm)			
	0	5	25	50
			Males	
No. of animals	20	19	20	7
Grams	17.8±2.2	18.2±2.4	21.5±2.7**	23.4±3.2**
Percent of body wt.	3.4±0.2	3.6±0.2	4.2±0.5**	5.1±0.7**
Percent of brain wt. ($\times 10^{-2}$)	9.8±1.0	8.0±1.1	9.4±1.2**	10.4±1.6**
			Females	
No. of animals	20	20	13	0
Grams	10.2±1.7	10.5±1.7	13.0±1.4**	-
Percent of body wt.	3.3±0.4	3.4±0.4	4.4±0.5**	-
Percent of brain wt. ($\times 10^{-2}$)	4.9±0.8	5.0±0.9	5.9±0.5**	-

**Significantly different from control value ($p \leq 0.01$).

TABLE 8. Selected Gross Lesions--Deaths and Unscheduled Sacrifices[a]

Organ/Lesion	Males Dietary Level (ppm)				Females Dietary Level (ppm)			
	0[a]	5	25	50	0[a]	5	25	50
No. of animals	(0)	(1)	(0)	(13)	(0)	(0)	(7)	(14)
Brain								
hemorrhage, basal	0	0	0	11	0	0	1	8
hemorrhage, cerebral	0	0	0	1	0	0	3	6
Intestine								
hemorrhage, duodenum	0	0	0	1	0	0	1	7
hemorrhage, jejunum	0	0	0	3	0	0	3	7
Thymus								
hemorrhage, petechial	0	0	0	8	0	0	3	7
Kidney								
congestion	0	0	0	5	0	0	1	5
Liver								
congestion, passive	0	1	0	13	0	0	7	12
Lung								
congestion	0	1	0	9	0	0	3	11

[a] No corresponding lesions were found in 20 animals/sex in the control group that were sacrificed at study termination.

TABLE 9. Histologic Lesions in Rats Fed Oxychlordane for 13 Weeks

Organ/Lesion	Males Dietary Level (ppm) 0	5	25	50	Females Dietary Level (ppm) 0	5	25	50
Brain								
Spongiosis, white matter	(20)[a]	(20)	(20)	(20)	(20)	(20)	(20)	(20)
	0	2	5*	6*	0	0	4*	14*
Spinal cord cervical	(20)[a]	(20)	(20)	(19)	(18)	(17)	(19)	(19)
Spongiosis, white matter	0	0	3	3	0	0	2	9
Liver	(20)[a]	(20)	(20)	(20)	(20)	(20)	(20)	(20)
Megalocytosis	0	6*	20*	19*	0	0	20*	14*
minimum/mild	0	6*	13*	5*	0	0	12*	12*
moderate/severe	0	0	7*	14*	0	0	8*	2
Hepatocyte vacuolization	1	2	3	8*	0	2	6*	2
Portal mononuclear infiltrates	12	0	11	7	2	0	0	0
Kidney	(20)[a]	(20)	(20)	(20)	(20)	(20)	(20)	(20)
Chronic progressive nephropathy	3	6	9*	7	0	0	2	1
Protein resorption droplets	7	8	12	9	0	0	0	0
Mineralization	0	0	0	0	0	2	0	4
Heart	(20)[a]	(1)	-	(20)	(20)	-	(7)	(20)
Myocarditis, chronic	7	-	-	2	2	-	-	1
Thymus	(20)[a]	(1)	-	(20)	(20)	(1)	(6)	(20)
Hemorrhage	4	1	-	8	7	1	2	4

[a]The numbers in parentheses are the number of tissues examined.

*Significantly different from control incidence ($p \leq 0.05$); analysis by these reviewers using the Fisher exact test.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

Oxychlordane administered to rats in the diet for 90 days resulted in toxic effects at all dose levels tested. Compound-related deaths occurred in 26 and 7 rats of the high- and mid-dose groups, respectively. Convulsions and hyperactivity, which were seen in the mid- and high-dose groups, were thought to be related to the spongiosis observed in animals in these groups after microscopic examination of the brain and spinal cord.

All the males and females that were sacrificed in mid- and high-dose groups exhibited megalocytosis of the liver. Other compound-related liver effects were observed, which included increases in absolute liver weight, liver weight relative to body weight, and liver weight relative to brain weight in the mid- and high-dose males and mid-dose females. Cholesterol levels were elevated at 6 and 12 weeks in the mid- and high-dose groups as well.

Compound-related effects were also observed in the low-dose group; however, the microscopic effects observed in this group were minimal. The no effect level for oxychlordane in males and females was stated to be slightly less than 5 ppm.

A quality assurance statement dated February 22, 1985, was present.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The design, conduct, and reporting of the study are acceptable and in accord with proposed guidelines. The summary data were supported by individual animal data.

The dates of clinical laboratory studies were not entered on the computerized sheets nor were the dates of body weight measurements; this caused some initial confusion to the reviewers. However, dates were entered on pathology sheets. In the report, week minus 1 is the week of initiation of dosing, study week 1 day 4 refers to day 11 of dosing and study week 0 refers to the first 7 days of dosing. Our tabulations refer to week 1 as the first week of dosing. For example, week 1 body weights are measured at day 7 whereas the report records body weights at day 7 as week 0.

Our assessment is that the conclusions of the study authors and their interpretation of toxicologic importance of data were correct, although we question whether a NOEL could be determined. If the histologic changes in liver of low-dose males are not considered of toxicologic importance because they are graded minimum, the NOEL could be set at 5 ppm. However, these changes were statistically significant in males receiving 5 ppm, and based on this finding it is our assessment that a NOEL was not achieved for this study.

Our assessment is that the highest dose tested caused excess mortality and the lowest dose tested could have been lower than 5 ppm so that a NOEL could be unequivocally set; therefore, we consider the study Core Minimum.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 5-14.