8-28-85

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

004635

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

AUG 28 1985

MEMORANDUM

SUBJECT: 6(a)(2) Data on Chlordane. Chronic mouse and rat studies for oncogenicity testing.
Acc. Nos. 252267, 254665 and 251815
Caswell No. 174

FROM: Henry Spencer, Ph.D. 8/27/85
Pharmacologist
Review Section 7
Toxicology Branch/HED (TS-767)

TO: Lois Rossi
Section Head
SRB/RD (TS-767)

THRU: Albin Kocialski, Head
Review Section 7
Toxicology Branch/HED (TS-767) 8/27/85 8/27/85

The rat and mouse studies have been reviewed and the results have been added to the files for chlordane.

Additionally, these studies have been transferred to the C.A.G. (Cancer Assessment Group), for possible reevaluation of chlordane's cancer risk.

HED:JOB-93891:Spencer:RD-13:Kendrick:898-1270:8/22/85:Del.9/2/85:Kim

Appendix A

Analysis of Test Material

APPENDIX A (1 page) and APPENDIX B (8 pages) contain product analysis and registration data submitted by Velsicol Chemical Corporation. These pages are not included with this copy of the review.

The results on clinical chemistry parameters are difficult to interpret because no toxicologic significance is apparent for decreases in activities of GOT, GPT, and ALP. The blood samples were collected in EDTA, centrifuged and analyzed within 1-2 days. It was not stated if the plasma samples were stored refrigerated or not. Potassium oxalate is the usual anticoagulant and samples are usually stored at -70° C if not analyzed immediately. No data was available on the normal serum enzyme levels in 130 week old rats; the aging process could possibly contribute to be decreased values. For both males and females at the 130 week sacrifice, the total protein values for all groups did not appear abnormal but the albumin/globulin (A/G) ratios were the inverse of what would be expected. For example, at 52 weeks, the A/G ratio ranged from 2.00-2.13 in males and 2.18-2.23 in females, whereas at 130 weeks the A/G ratios ranged between 0.67 and 0.76 in males and 0.90-0.94 in females. Clinical laboratory studies were not conducted at weeks 78 or 104, and only 8 animals/sex/group were examined at 26 and 52 weeks rather than 12. All surviving animals at 130 weeks were included in the clinical laboratory examination.

16. CBI APPENDIX:

Appendix A, Methods and Materials; CBI Vol I pp. 8-18.

EPA: 68-01-6561
TASK: 87
February 14, 1985

DATA EVALUATION RECORD

CHLORDANE

Chronic Feeding Study in Mice

STUDY IDENTIFICATION: Inui, S., Yamazaki, K., Yonemura, T., et al. Twenty-four month chronic toxicity and tumorigenicity test in mice by chlordane technical. (Unpublished report prepared by Research Institute for Animal Science in Biochemistry and Toxicology, Japan for Velsicol Chemical Corporation Chicago, IL; dated December 1, 1983.) Accession Nos. 254665 and 251815.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 2-14-85

Typo - p. 3

1. CHEMICAL: Chlordane technical; 1, 2, 4, 5, 6, 7, 8, 8-octachloro-2, 3, 3a, 4, 7, 7a-hexahydro-4,7-methanoindane and related compounds.

2. TEST MATERIAL: Chlordane technical, purity 100%; Lot No. B-9129A.

3. STUDY/ACTION TYPE: Chronic feeding study in mice.

4. STUDY IDENTIFICATION: Inui, S., Yamazaki, K., Yonemura, T., et al. Twenty-four month chronic toxicity and tumorigenicity test in mice by chlordane technical. (Unpublished report prepared by Research Institute for Animal Science in Biochemistry and Toxicology, Japan for Velsicol Chemical Corporation Chicago, IL;) dated December 1, 1983. Accession Nos. 254665 and 251815.

5. REVIEWED BY:

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7. CONCLUSIONS:

A. Chlordane was oncogenic in male ICR mice causing an increased incidence of hepatocellular adenomas and hepatic hemangiomas when fed for two years at a dietary concentration of 12.5 ppm. Non-neoplastic changes were found in the liver: hepatocellular

swelling and necrosis in males receiving 5 or 12.5 ppm, fatty degeneration in males receiving 12.5 ppm, and hepatocyte swelling in females receiving 5 and 12.5 ppm. There was an increase in mean weight of livers in both male and female mice receiving chlordane accompanying the histologic changes. The NOEL for chlordane technical in mice based on non-neoplastic changes in the liver was 1 ppm and the LEL was 5 ppm.

Core evaluation: minimum mrk 8/30/85

8. RECOMMENDATIONS: Not applicable.

9. BACKGROUND:

The rationale for choice of dose levels was based on a 4-week pilot feeding study in which the lowest dose used, 10 ppm, caused hepatocyte swelling. Choice of the lowest dose was based on an 18-month oral carcinogenicity study in mice provided by Velsicol in which changes in the liver were evident in the 5 ppm group.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: Not applicable.

11. A. Materials and Methods:

The test material was chlordane technical. Analysis of Lot B-9129A is provided in Appendix A. Groups of 80 male and 80 female ICR mice were fed chlordane in the diet at levels of 0, 1, 5, or 12.5 ppm for two years. Animals were observed twice daily for toxic signs. Body weights were measured weekly for 26 weeks and twice weekly thereafter. Food consumption and water intake were measured weekly for 8 cages (5 mice/cage). Urinalysis, hematology, and blood chemistry were measured on 8 mice/sex/group at 52 weeks and on all survivors at week 104. Organ weights were taken on 8 animals/sex/group sacrificed at week 52 and on all survivors at study termination. Complete gross examination and histopathologic evaluation of a complete set of tissues were performed on all animals in the study.

Data were analyzed statistically. The word "significant" in this evaluation was used to imply a statistical connotation.

B. Protocol:

See Appendix B for protocol details.

12. REPORTED RESULTS:

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Analysis of Diets: Chlordane in the diet was analyzed at intervals ranging from 3 to 6 months. The means and standard deviations for the study were:

Nominal (ppm)		Analytical (ppm)	Range (ppm)
1	-	0.76 ± 0.065	0.7 - 0.83
5	-	3.628± 0.404	2.99 - 4.0
12.5	-	9.32 ± 1.43	7.24 - 10.7

Homogeneity and stability of chlordane in the diets were not provided. Diets were prepared every 3 months.

Observations and Mortality: There were no unusual clinical signs that were related to dosing. Clinical observations were undetailed and entered on the pathology charts. There was no effect of dosing on survival. Mortality data are summarized in Table 1. Survival at 18 months ranged from 75 to 78 percent in males and 78 to 85 percent in females. Survival ranged from 38 to 50 percent at 24 months except in the group of males receiving 1 ppm where survival was 26 percent.

Body Weights: The body weights of mice "were generally unaffected by treatment". Table 2 summarizes body weights at selected intervals. There was a trend towards increased mean body weights in females at 1 ppm compared to controls.

Food and Water Consumption: Males fed chlordane had an increased food consumption when compared with controls during weeks 96 and 104 (Table 3). Food consumption was increased in all dosed groups of females at week 104. Food efficiency was similar in control and dosed groups of mice. The report stated that there was a "mild treatment related trend to increased water intake in males but not in females."

Hematology: A significant increase in hematocrit and hemoglobin were noted in males receiving 12.5 ppm at 52 weeks when compared to controls, but the values were considered within the normal range. These parameters were similar in all groups of males and females at study termination. Fluctuations in differential white cell counts and platelet counts noted at study termination were not related to dosing and were considered within the normal limits.

Clinical Chemistry: The authors stated that there was a tendency towards elevated GOT and GPT in both males and females receiving chlordane. Some animals had abnormally high values which correlated with swelling of hepatocytes observed on histopathologic examination and with increased liver weights in these individual animals. Table 4 summarizes mean values for GOT and GPT.

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EPA: 68-01-6561
TASK: 87
February 14, 1985

DATA EVALUATION RECORD

TECHNICAL CHLORDANE

Chronic Feeding Study in Rats

STUDY IDENTIFICATION: Yonemura, T., Takamura, F., and Takahashi, Y. Thirty-month chronic toxicity and tumorigenicity test in rats by chlordane technical. (Unpublished study by Research Institute for Animal Science in Biochemistry and Toxicology, Japan for Velsicol Chemical Corp., Chicago, IL; dated December 1, 1983. Accession No. 252267.)

APPROVED BY:

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

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Date: _____

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1. CHEMICAL: Chlordane technical.

2. TEST MATERIAL: Chlordane technical, 100% pure.

3. STUDY/ACTION TYPE: Chronic feeding study in rats.

4. STUDY IDENTIFICATION: Yonemura, T., Takamura, F., and Takahashi, Y. Thirty-month chronic toxicity and tumorigenicity test in rats by chlordane technical. (Unpublished study by Research Institute for Animal Science in Biochemistry and Toxicology, Japan for Velsicol Chemical Corp., Chicago, IL; dated December 1, 1983. Accession No. 252267.)

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Date: 8/20/85

Albin Kocialski, Ph.D.
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7. CONCLUSIONS:

A. Under the conditions of the study, chlordane technical caused a significant increase in the incidence of benign hepatocellular tumors when fed at a level of 25 ppm in the diet to male F344 rats for 130 weeks. There was also a significant increase in non-neoplastic lesions of the liver in both male and female rats. There was an increased incidence of hepatocellular swelling in males receiving 1, 5, or 25 ppm and in females receiving 25 ppm chlordane technical compared to controls. In addition, there was a significant increase in hepatocellular necrosis in males receiving 1 or 25 ppm but no corresponding effect in females. The histologic changes in the liver were accompanied by increased liver weights in males at 130 weeks (5 and 25 ppm groups) and females receiving 25 ppm at weeks 26 and 52. A NOEL and LOEL for chronic toxicity in females based on non-neoplastic changes in the liver are 5 ppm and 25 ppm; the LOEL in males is 1 ppm.

Core Evaluation: minimum 8/20/85

8. RECOMMENDATIONS: Not applicable.

9. BACKGROUND:

The dose levels chosen were based on the results of a pilot study in which groups of 5 male and 5 female Fischer 344 rats were fed diets containing 0, 50, 100, 200, 400, or 800 ppm technical chlordane for 4 weeks. Hepatocellular swelling and fatty degeneration in the liver were found in both male and female rats at 50 ppm, the lowest dose tested. A 1 ppm group was set as the no effect level based on an 18-month study in mice (provided by Velsicol) in which changes in the liver were evidenced at 5 ppm chlordane.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: Not applicable.

11. MATERIALS AND METHODS (PROTOCOLS): Groups of 80 male and 80 female Fischer 344 rats (Charles River, Japan K.K.) were fed diets containing chlordane at levels of 0, 1, 5, or 25 ppm, for 130 weeks. Animals were housed in groups of 3 per cage, and observed daily for toxic signs. Body weights were measured weekly for 26 weeks and biweekly thereafter. Food consumption and water intake were measured on 8 cages/sex/group. Clinical laboratory studies were performed on 8 animals/sex/group at weeks 26 and 52, and on all survivors at week 130. Organ weights were measured on 8 animals/sex/group sacrificed by design at weeks 26 and 52, and on all survivors. All animals that were sacrificed or died during the study were examined grossly. All tissues and macroscopic lesions were examined microscopically.

Statistical analysis employed the Student's "t" test. Incidence of pathologic lesions were analyzed by the chi-square test with Yates correction on the Fisher exact test. The word "significant" in this evaluation was used to imply a statistical connotation.

Protocol: See Appendix A for detailed protocol.

12. REPORTED RESULTS:

Dietary Analysis: The amount of chlordane in the diets was analyzed at 8 intervals throughout the study. The mean levels were as follows (Table 1):

TABLE 1. Dietary Analysis of Chlordane

Nominal (ppm)	Analyzed Mean ±S.D. (ppm)	Analyzed Range (ppm)
1	0.92 ± 0.20	0.7 - 1.39
5	4.37 ± 0.79	3.60 - 6.32
25	23.01 ± 3.26	18.60 - 29.62

Data on homogeneity in the diet and stability were not provided.

Observations and Mortality: No toxic signs related to dosing with chlordane were observed during any part of the study. Very brief entries of clinical observation were entered in the pathology tabulation for individual animals. Table 2 summarizes mortality and percent survival data at selected intervals during the study. There were no compound-related trends in mortality.

Body Weights: Dosed males and females showed occasional differences in mean values when compared to controls, but there were no effects considered to be related to dosing. The mean weights of high-dose females were lower than controls at several weighings, but the differences were in the range of 2-3 percent of body weight (Table 3).

Food and Water Consumption: There were sporadic fluctuations in mean food consumption during the study but no dose-related trends. The chemical intake throughout the study was calculated as 0.045, 0.229, and 1.175 mg/kg/day in the 1, 5, and 25 ppm groups of males and 0.055, 0.273, and 1.409 mg/kg/day in the same groups of females, respectively. There were no marked differences in water consumption between the dosed and control groups.

TABLE 2. Mortality and Percent Survival in Rats Fed Chlordane for 130 Weeks

Group[a]/Dose (ppm)	Mortality and (Percent Survival) at Week					
	26	52	78	104	118	130
			MALES			
0	0(100)	0(100)	1(98)	20(69)	37(42)	52(19)
1	0(100)	0(100)	2(97)	17(73)	28(56)	44(31)
5	0(100)	0(100)	2(97)	16(75)	33(48)	53(17)
25	0(100)	0(100)	2(97)	15(77)	39(39)	55(14)
			FEMALES			
0	0(100)	0(100)	1(98)	22(66)	31(52)	41(36)
1	0(100)	2(97)	2(97)	16(75)	26(59)	40(38)
5	0(100)	1(98)	1(98)	17(73)	26(59)	36(44)
25	0(100)	0(100)	3(95)	14(78)	24(63)	40(38)

[a] Sixty-four animals/sex/group; animals killed by design at 26 and 52 weeks were not included in the mortality calculations.

TABLE 3. Selected Mean Body Weights of Rats Fed Chlordane Technical for 130 Weeks

Group/Dose (ppm)	Body Weights in Grams at Week					
	26	52	78	104	118	130
			MALES			
0	362.6 ±20.3	419.7 ±22.9	435.9 ±27.3	402.3 ±51.0	356.8 ±54.8	314.5 ±52.7
1	364.7 ±19.4	426.1 ±22.9	446.7 ±39.8	435.1** ±31.5	380.8 ±50.7	335.5 ±44.6
5	353.7* ±20.6	414.5 ±26.4	439.3 ±29.1	414.9 ±41.3	375.9 ±63.6	357.4 ±56.2
25	360.0 ±17.7	421.6 ±20.6	441.1 ±25.8	407.2 ±43.3	380.6 ±44.9	355.1 ±74.2
			FEMALES			
0	201.5 ±11.1	233.0 ±16.6	280.0 ±34.6	301.6 ±40.4	296.4 ±42.1	288.1 ±42.8
1	200.3 ±8.3	236.2 ±14.1	285.6 ±30.7	310.1 ±38.0	297.7 ±46.8	294.6 ±41.5
5	196.5** ±10.1	233.1 ±18.6	274.1 ±36.9	301.4 ±32.7	288.7 ±37.3	271.5 ±46.2
25	196.1** ±9.1	230.1 ±14.1	272.0 ±30.4	292.8 ±33.2	276.6 ±45.4	267.4 ±36.0

* Statistically different from control value ($p < 0.05$).
** Statistically different from control value ($p < 0.01$).

Hematology: There were no compound-associated effects on hematology parameters. Although there were some slight fluctuations in values, all were within the normal range.

Clinical Chemistry: There was a slight increase in mean bilirubin values in males receiving 5 and 25 ppm at week 130; GOT, GPT, and ALP levels were decreased in males receiving 25 ppm at weeks 26 and 52 but the report stated that the values were not abnormal. GOT and LDH were decreased and cholesterol increased at 130 weeks in females receiving 25 ppm. LDH, GOT, and GPT were also decreased at 26 weeks in females receiving 25 ppm. None of these changes were regarded by the authors as abnormal.

Urinalyses: There were no compound-related effects on urinalysis parameters. At 130 weeks there was an increase on urinary protein and occult blood in all groups of ~~mice.~~ rats. This was considered due to aging.

Organ Weights: There was a significant increase in the weight of livers in females receiving 25 ppm at weeks 26 and 52 but not at week 130 (Table 4). Liver weight was significantly increased in males receiving 5 and 25 ppm at week 130 but not at the 26 or 52 week sacrifice. Weight changes in other organs were not consistent or dosed related.

Gross Pathology: At necropsy there was swelling, paleness, atrophy, and mass formations in various organs but the incidences of these findings were similar among groups. Enlargement of the liver was noted in 19 control males and 19, 26, and 32 males at dosed 1, 5, and 25 ppm, respectively. There was a slight increase in subcutis nodules or masses in dosed males (8 in controls and 12 in each dosed group).

Histopathology: Table 5 summarizes the neoplastic lesions found in animals that died, were sacrificed moribund, or were sacrificed at 30 months. There was a significant increased incidence of adenomas of the liver in males receiving 25 ppm as compared to controls but no corresponding effect in females. All of these tumors were found after 104 weeks (mean time to tumor death was 121.8 weeks). There was also a significant increase in fibroadenomas of the mammary gland in females receiving 1 ppm as compared to controls but no significant increase at 5 or 25 ppm. The incidences of all other tumors were similar among groups except that the total number of tumors of the skin and subcutis combined was significantly higher in males receiving 25 ppm than in controls.

Non-neoplastic lesions occurring frequently are summarized in Table 6. There was a dose-related increase in the incidence of hepatocellular swelling and hepatocellular necrosis in male rats. When compared to controls, the incidence of hepatocellular swelling was significantly increased in all dosed males and the incidence of hepatocellular necrosis was significantly increased in males receiving 1 and 25 ppm. In females, the incidence of hepatocellular swelling was significantly

TABLE 4. Absolute and Relative Liver Weights in Rats Fed Diets Containing Chlordane

	Dose (ppm)			
	0	1	5	25
		26-Week Sacrifice		
Males				
grams	8.54±0.85[a]	8.33±0.32	8.53±0.68	9.00±0.55
percent	2.48±0.36	2.36±0.09	2.46±0.10	2.54±0.10
Females				
grams	4.62±0.29	4.42±0.32	4.43±0.21	5.02±0.18*
percent	2.28±0.12	2.29±0.11	2.32±0.14	2.57±0.06*
		52-Week Sacrifice		
Males				
grams	10.75±0.73	9.86±0.81*	10.05±0.89	10.81±0.78
percent	2.49±0.11	2.32±0.11*	2.42±0.14	2.59±0.07
Females				
grams	5.27±0.28	5.23±0.30	5.41±0.45	5.77±0.33*
percent	2.38±0.12	2.29±0.14	2.33±0.10	2.55±0.18
		130-Week Sacrifice		
Males				
grams	10.44±1.56	12.38±5.14	13.33±3.22*	13.30±2.60*
percent	3.48±0.58	4.00±2.21	4.07±1.25	4.11±0.84
Females				
grams	8.28±1.85	8.04±1.61	8.83±2.83	8.46±1.05
percent	3.10±0.70	2.92±0.73	3.55±1.43	3.40±0.40

[a]Standard deviation.
*Significantly different from control value ($p < 0.05$).

TABLE 5. Neoplastic Lesions Found in Rats Fed Chlordane for 130 Weeks[a]

Organ/Finding	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	25	0	1	5	25
Spleen	(64)[b]	(64)	(64)	(64)	(64)	(64)	(64)	(64)
hemangioendothelioma	1	0	0	2	0	0	0	0
Hematopoietic system	(64)[b]	(64)	(64)	(64)	(64)	(64)	(64)	(64)
lymphocytic leukemia	6	5	7	10	4	2	5	4
mononuclear cell leukemia	34	30	34	35	22	16	19	15
Skin	(61)[b]	(63)	(61)	(64)	(64)	(64)	(64)	(64)
squamous cell papilloma	0	0	2	1	1	0	0	0
keratoacanthoma	2	1	2	2	0	0	2	0
squamous cell carcinoma	0	0	2	1	0	0	0	2
Skin-subcutis	(64)[b]	(64)	(64)	(63)	(64)	(64)	(64)	(64)
fibroma	4	5	9	7	1	1	4	1
fibrosarcoma	1	0	0	2	0	0	1	1
rhabdomyosarcoma	0	0	0	2	0	0	0	1
leiomyosarcoma	0	3	0	1	0	3	0	0
hemangiomyofibroma	1	3	1	1	0	1	4	2
Skin/Subcutis Total	9	15	17	19*	5	7	11	9
Mammary gland					(44)[b]	(40)	(45)	(44)
fibroadenoma					3	12*	6	6
adenoma					3	3	4	6
adenocarcinoma					0	0	2	0
Liver	(64)[b]	(64)	(64) 4.6%	(64) 14%	(64)	(64)	(64)	(64)
hepatocellular adenoma	1	1	3	9*	0	2	0	0
mesenchymoma (B)	0	2	1	0	0	0	0	0
mesenchymoma	0	1	0	2	0	0	0	0
Pancreas	(62)[b]	(62)	(64)	(63)	(63)	(64)	(62)	(64)
islet cell adenoma	3	1	2	1	1	2	0	0
Lung	(64)[b]	(64)	(64)	(64)	(64)	(64)	(64)	(64)
adenoma	3	3	4	1	0	1	0	1
Urinary bladder	(56)[b]	(56)	(58)	(58)	(64)	(63)	(58)	(61)
papilloma	0	0	0	1	1	1	2	0
Ovary					(64)	(64)	(64)	(64)
granulosa cell tumor					0	0	2	0

TABLE 5. Neoplastic Lesions Found in Rats Fed Chlordane for 130 Weeks[a] (Continued)

Organ/Finding	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	25	0	1	5	25
Testis	(64)[b]	(64)	(64)	(64)				
interstitial cell tumor	61	59	63	60				
Uterus					(64)[b]	(64)	(64)	(64)
endometrial stromal polyp					11	6	8	8
leiomyoma					1	2	1	1
hemangioma					1	2	2	0
Pituitary	(63)[b]	(62)	(63)	(63)	(64)	(64)	(63)	(62)
adenoma, anterior	7	6	1	4	26	21	26	29
Thyroid	(64)[b]	(63)	(60)	(65)	(63)	(63)	(62)	(63)
C-cell adenoma	8	8	7	4	7	8	6	3
C-cell carcinoma	10	10	12	10	9	12	11	8
Adrenal	(64)[b]	(63)	(64)	(64)	(64)	(64)	(63)	(62)
pheochromocytoma (B)	15	13	13	15	5	9	13	8
pheochromocytoma (M)	0	1	0	0	2	1	0	1
Abdominal cavity	(64)[b]	(64)	(64)	(64)	(64)	(64)	(64)	(64)
mesothelioma (M)	1	5	2	4	0	1	0	0

[a] Does not include animals sacrificed at 26 and 52 weeks. Tabulation is only of primary tumors that occurred in at least two animals of one group. Prepared by our reviewers.

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

TABLE 6. Number of Frequent Non-neoplastic Lesions in Rats Fed Chlordane[a]

Organ/Lesion	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	25	0	1	5	25
Liver	(64)[b]	(64)	(64)	(64)	(64)	(64)	(64)	(64)
hepatocellular swelling	5	15*	14*	42**	7	2	8	38**
hepatocellular necrosis	3	13*	11	27**	0	0	1	1
hepatocellular fatty degeneration	26	15*	19	22	20	16	19	2
bile duct proliferation	57	59	60	64	23	31	31	23
focal hepatocellular hyperplasia	5	7	3	11	5	10	1	1
hepatocellular atrophy	13	9	11	11	6	8	14	8
focal telangiectasis	23	24	20	26	0	0	1	2
congestion	4	5	10	12	7	5	3	5
Spleen	(63)[b]	(64)	(64)	(64)	(63)	(64)	(64)	(64)
fibrosis	6	16*	11	7	6	5	1	1
congestion	4	14*	10	10	8	5	9	13
increased extramedullary hematopoiesis	3	6	6	5	5	16*	10	7
Lung	(64)[b]	(64)	(64)	(64)	(63)	(64)	(64)	(63)
hemorrhage	5	7	6	13*	1	2	2	2
Adrenal	(64)[b]	(63)	(64)	(64)	(63)	(64)	(63)	(64)
sinus dilation	7	19*	15	16*	16	26	21	21
Lacrimal gland	(64)[b]	(62)	(63)	(63)	(61)	(63)	(64)	(63)
increased secretion	2	8	11*	5	6	4	6	5
Stomach	(64)[b]	(64)	(64)	(64)	(63)	(64)	(63)	(63)
hyaline thickening	3	9	13*	4	1	2	2	1

[a]The animals sacrificed by design at weeks 26 and 52 are not included.
[b]Number of tissues examined.
*Significantly different from control value ($p < 0.05$).
**Significantly different from control value ($p < 0.001$).

higher in females receiving 25 ppm than in controls. There was also an increase in focal hepatocellular hyperplasia in males receiving 25 ppm but the increase was not significantly different compared to controls. Most of these lesions of the liver occurred after 78 weeks in the study. Findings in the livers of groups sacrificed at 26 and 52 weeks are summarized in Table 7. Other non-neoplastic lesions whose incidence was increased in dosed animals compared to controls are summarized in Table 6. There was no dose-related trend; almost all the lesions occurred after week 78.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

Chlordane was "thought to be positive for tumorigenicity" because the incidence of hepatic adenomas was significantly increased in males in the 25 ppm group. The historical incidence of this tumor in F344/CRJ mice for the testing laboratory was 2.5 percent in males and 2.3 percent in females (CBI Appendix 29-4). The control incidence in this study was 1.6 percent. The incidence in another study by the testing laboratory was 9.6 percent in 42 males and 19.2 percent in 48 females; these rats were on diet "over 105 weeks" (CBI Appendix 29-6). ie 92 yrs.

An independent review of the liver histopathology was included with the submission of the report. The data are summarized in Table 8.

Three of the neoplasms of the liver identified as adenomas by the report authors were diagnosed as carcinomas by the reviewing pathologist. In addition 3 neoplasms were found that were not diagnosed by the testing laboratory. The adenomas would be called neoplastic nodules in the U.S. It was noted that only 2 slices of liver, one from the median lobe and one from the left lobe, were taken from rats without grossly observed tumors, and it was stated that if more sections were taken for examination it is possible that the number of neoplasms would have increased. In the opinion of the reviewing pathologist, the small increase in benign liver neoplasms was considered as weak evidence for the oncogenicity of chlordane in rats.

The increased incidence of mammary fibroadenomas in females receiving 1 ppm was not considered to be compound/dose related because mammary fibroadenomas were absent in females dosed at higher levels.

Non-neoplastic changes in the liver of both males and females were increased in dosed animals compared to controls. The principal changes consisted of hepatocellular swelling and necrosis. The liver changes were accompanied by an increase in liver weights in males receiving 5 and 25 ppm at 130 weeks and in females receiving 25 ppm at weeks 26 and 52.

TABLE 7. Liver Lesions in Rats Fed Chlordane for 26 or 52 Weeks[a]

Lesion	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	25	0	1	5	25
26 Weeks								
Hepatocellular fatty degeneration	0	0	0	1	0	0	1	0
Focal necrosis	0	0	0	0	0	1	0	0
Bile duct proliferation	0	0	0	1	1	0	0	1
52 Weeks								
Hepatocellular fatty degeneration	0	2	0	1	0	0	0	0
Hepatocellular swelling	0	0	0	2	0	1	0	3
Focal necrosis	0	0	1	1	0	1	0	1
Bile duct proliferation	0	0	0	0	0	0	1	0
Small granuloma	0	0	0	0	0	0	1	2

[a]Livers from 8 rats/group were examined.

TABLE 8. Liver Neoplasms in Male Rats Fed Chlordane[a]

Neoplasm	Dose (ppm) 0	1	5	25
Adenoma	1[b]	1	4	8[b]
Carcinoma	1[b]	0	0	2[b]
Total neoplasms	2	1	4	10

[a] Pathology by Gary M. Williams, M.D., dated March 9, 1984.

[b] One animal had both an adenoma and carcinoma.

Based on non-neoplastic changes in the liver a no effect level in males and females was stated to be 1 and 5 ppm, respectively, and the minimum certain toxic level in both sexes was 25 ppm.

A quality assurance statement dated December 1, 1983 was present.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We agree with the conclusions of the study authors that under the conditions of the study, chlordane caused oncogenicity in F344 rats. This assessment is based on the significant increase in the incidence of adenomas of the liver in males receiving 25 ppm compared to controls. The reevaluation of liver histopathology by the registrant's pathologist changed the diagnosis of some the liver tumors from adenoma to carcinoma, and 3 additional liver neoplasms were diagnosed. However, these interpretations do not change the authors' conclusions. When the histopathology data was statistically analyzed, the authors did not censor the animals sacrificed at 26 and 52 weeks; however, this does not change the level of statistical significance of the findings. Our summary tabulations of histologic lesions in Tables 5 and 6 censors data for animals at 26 and 52 weeks, and we present interim sacrifice findings on the liver in Table 7. In addition, the data we present in Tables 5 and 6 indicate the number of tissues examined histopathologically; this data was not presented in the summary tables of the authors' final report.

All of the liver tumors occurred in males that died between week 104 and 130 of the study. The mammary fibroadenomas were all found after week 78 of the study. The majority of the the non-neoplastic liver findings were observed after week 104 of the study.

We agree with the author's conclusions that there were no effects of dosing which were of toxicologic importance for mortality, food consumption, water consumption, or urinalysis.

TABLE 1. Mortality and Percent Survival in Mice Fed Chlordane Technical for 24 Months

Group[a]/Dose(ppm)	Mortality (and Percent Survival) at Week				
	13	26	52	78	104
Males					
0	0(100)	0(100)	4(94)	18(75)	44(39)
1	0(100)	2(97)	9(88)	18(75)	53(26)
5	0(100)	3(96)	8(89)	16(78)	44(39)
12.5	0(100)	2(97)	8(89)	18(75)	45(38)
Females					
0	0(100)	2(97)	6(92)	17(76)	42(42)
1	0(100)	0(100)	8(89)	20(72)	40(44)
5	0(100)	0(100)	8(89)	15(79)	36(50)
12.5	0(100)	0(100)	4(94)	12(83)	38(47)

[a] 72 animals/group; animals scheduled for 52 week sacrifice were not included in mortality calculations, authors' statement.

TABLE 2. Selected Mean Body Weights for Mice Fed Chlordane Technical for 24 Months

Group/Dose(ppm)	Body Weights (g) at week					
	13	26	52	78	104	Termination
Males						
0	41.1 ±4.2[a]	44.5 ±5.4	47.4 ±6.3	48.0 ±7.4	42.3 ±7.0	40.1 ±6.39
1	41.1 ±3.3	44.4 ±5.0	47.5 ±5.9	47.0 ±5.4	43.3 ±4.8	39.6 ±4.54
5	39.0** ±3.2	42.5* ±3.9	46.4 ±5.2	46.0 ±6.2	42.9 ±4.6	38.2 ±4.15
12.5	40.7 ±3.6	43.9 ±4.3	47.3 ±5.6	47.1 ±5.6	42.9 ±5.4	41.4 ±6.01
Females						
0	30.1 ±2.7	32.4 ±3.8	37.3 ±6.2	38.3 ±6.7	34.9 ±6.8	32.9 ±6.97
1	30.6 ±2.5	33.1 ±3.6	39.4* ±6.2	40.9 ±8.2	39.7* ±6.6	37.9** ±6.0
5	29.8 ±2.6	32.3 ±3.9	37.8 ±7.1	40.1 ±8.2	36.7 ±6.8	34.9 ±5.98
12.5	30.7 ±2.5	32.8 ±3.1	37.9 ±5.8	39.1 ±6.7	37.7 ±5.9	36.3 ±5.71

[a]Standard deviation.

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

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

TABLE 3. Selected Mean Food Consumption in Male Mice Fed Chlordane Technical for 24 Months

Group/Dose(ppm)	Gram of Food/Mouse/Day at Week						
	92	94	96	98	100	102	104
0	4.9 ±0.41[a]	5.4 ±0.65	4.8 ±0.40	4.5 ±0.40	4.5 ±0.64	4.5 ±0.47	4.6 ±0.30
1	4.8 ±0.73	5.4 ±0.69	5.8 ±0.94	5.5* ±0.88	5.2 ±0.39	5.4* ±0.72	5.4* ±0.64
5	5.0 ±0.40	5.8 ±0.37	5.7* ±0.46	6.0** ±0.59	5.8** ±0.51	5.8* ±1.06	5.8* ±0.79
12.5	4.9 ±0.61	4.8 ±0.50	6.1** ±0.59	6.0*** ±0.30	6.0** ±0.39	5.8** ±0.43	6.5*** ±0.70

[a]Standard deviation.

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

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

***Significantly different from control value ($p \leq 0.001$).

TABLE 4. Serum Levels of Glutamic Oxaloacetic Transaminase (SGOT) and Glutamic Pyruvic Transaminase (SGPT) in Mice Fed Chlordane Technical

	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	12.5	0	1	5	12.5
SGOT (IU/L)								
52 weeks	77 ±26[a]	87 ±33	92 ±57	71 ±22	107 ±35	134 ±54	150* ±22	213 ±223
104 Weeks	151 ±227	110 ±59	127 ±61	215 ±136	116 ±63	123 ±149	105 ±58	112 ±75
SGPT (IU/L)								
52 Weeks	47 ±41	52 ±59	50 ±50	35 ±10	33 ±13	47 ±43	40 ±18	113 ±200
104 Weeks	102 ±181	90 ±73	135 ±96	308* ±195	42 ±27	56 ±60	50 ±33	90 ±103

[a]Standard deviation.

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

Urinalysis: There were no changes in urinary parameters related to dosing.

Organ Weights: The mean liver weight was significantly increased for the eight males receiving 12.5 ppm chlordane that were sacrificed at 52 weeks, and the liver-to-body weight ratios were significantly increased for all dosed groups of males compared to controls. At terminal sacrifice (104 weeks), the mean weights and organ-to-body weight ratios of the liver were significantly increased in both males and females receiving 12.5 ppm when compared to controls (Table 5). In addition, the liver-to-body weight ratios of females receiving 1 and 5 ppm chlordane were significantly greater than in controls.

Gross Pathology: Kidney, spleen, testis or ovaries, and seminal vesicles or uterus exhibited swelling, paleness, atrophy, and masses in several mice; however, the incidence of these findings were similar among all groups of males or females. There was an increased incidence of masses in the liver of dosed males; 14/79 in controls and 14/79, 18/80, and 32/80 at 1, 5, and 12.5 ppm, respectively.

The incidence at 12.5 ppm was significantly greater than in controls. There was a non-significant increase in liver masses in dosed females, 1/80 in controls and 2/80, 4/79, and 4/80 at 1, 5, and 12.5 ppm, respectively.

Histopathology: A significant increase in the incidence of hepatocellular adenoma and hemangioma of the liver were found when males receiving 12.5 ppm chlordane were compared with controls. These tumors were found between months 19 and 24 of the study. The hemangiomas were stated to be associated with the adenomas. There was no increase in hepatic tumors in female mice dosed with chlordane. Incidence of neoplastic lesions are summarized in Table 6.

Lesions occuring in the livers of mice at the 12-month sacrifice are summarized in Table 7. There was a dose-related increased incidence of hepatocellular swelling and degeneration, fatty degeneration and necrosis in the livers of males with a less distinct trend in females. The incidence of non-neoplastic changes in the liver for mice that died, were sacrificed moribund, or sacrificed at 104 weeks are presented in Table 8. There was a significant increase in the incidence of male mice with hepatocellular swelling and degeneration and with necrosis at dose levels of 5 and 12.5 ppm and a significant increase in fatty degeneration at 12.5 ppm. For females, there was an increased incidence of hepatocellular swelling at doses of 5 and 12.5 ppm chlordane when compared with controls.

There were no apparent dose-related increases in non-neoplastic lesions at sites other than the liver (Table 9).

TABLE 5. Mean Liver Weights and Liver-to-Body Weight Ratios in Mice Fed Chlordane

Dietary Level (ppm)	Males/Liver		Females/Liver	
	Weight(g)	Ratio(%BW)	Weight(g)	Ratio (%BW)
12-Month Sacrifice				
0	1.60±0.24	3.39±0.38	1.52±0.24	3.87±0.62
1	1.87±0.28	3.95±0.50*	1.52±0.34	3.66±0.56
5	1.67±0.19	3.97±0.29**	1.52±0.16	3.60±0.50
12.5	1.91±0.23*	4.40±0.34***	1.67±0.19	4.41±0.68
24-Month Sacrifice				
0	1.92±0.54	4.8±1.30	1.56±0.42	4.77±1.00
1	2.33±1.15	5.81±2.65	1.90±0.57*	5.04±1.36
5	2.48±1.48	6.30±3.63	1.78±0.39*	5.14±0.95
12.5	3.99±2.02**	9.82±4.99***	2.24±0.96***	6.13±2.09**

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

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

***Significantly different from control value ($p \leq 0.001$).

TABLE 6. Neoplastic Lesions in Mice Fed Chlordane Technical for Two Years[a]

Organ/Lesion	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	12.5	0	1	5	12.5
Liver	(71)[a]	(71)	(72)	(72)	(72)	(72)	(71)	(72)
hepatocellular adenoma	13	13	15	28**	1	1	3	1
hepatocellular adeno-carcinoma	3	3	7	9	0	0	0	1
hemangioma	~~4~~	~~1~~	~~8~~	~~14~~*	0	2	1	0
	16	16	22	37				
Lung	(71)	(71)	(72)	(72)	(72)	(72)	(71)	(72)
adenoma	1	2	4	3	1	0	2	5
adenocarcinoma	11	12	16	11	12	8	9	10
Hematopoietic	(71)	(71)	(72)	(72)	(72)	(72)	(71)	(72)
lymphoma, lymphatic	7	7	6	7	18	20	9	11
lymphoma, reticular cell	1	2	0	0	2	3	2	1
myeloid leukemia	4	0	1	0	0	0	0	0
Skin	(71)	(71)	(72)	(72)	(72)	(72)	(71)	(72)
adenoma	0	2	1	0	2	1	0	1
fibroma	4	1	3	0	0	0	0	0
rhabdomyoma	0	0	0	1	0	0	0	0
fibrosarcoma	1	1	3	0	0	0	0	0
fibrous histiocytoma	1	7	0	0	3	0	0	2
prickle cell tumor	0	0	0	0	0	1	0	0
chondroma	0	0	0	0	0	0	0	1
Uterus					(72)	(72)	(71)	(71)
leiomyoma					3	0	3	4
leumangioma					1	1	2	2
Mammary gland					(71)	(72)	(71)	(72)
adenocarcinoma					4	2	5	3
adenoma					0	2	0	0
Pituitary	(72)	(69)	(69)	(72)	(71)	(70)	(70)	(70)
adenoma	0	0	0	1	1	1	2	1
Lacrimal gland								
adenoma	0	1	0	4	0	0	1	0

[a] Neoplasms in mice sacrificed at 52 weeks were not included in this tabulation. Also a solitary tumor in group was not tabulated.

[b] Number in parenthesis is the number of tissues examined histologically.

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

** Statistically different from control value ($p < 0.01$).
Analysis by the registrant using chi-square with Yates correction.

TABLE 7. Liver Lesions in Mice Fed Chlordane Technical For 52-Weeks[a]

Lesion	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	12.5	0	1	5	12.5
No. examined	8	8	8	8	8	8	8	8
Hepatocellular swelling and degeneration	0	1	3	8***	0	0	0	2
Fatty degeneration	0	1	1	5**	2	0	0	0
Necrosis	1	1	2	5	1	2	2	3
Adenoma	0	1	0	0	0	0	0	0

[a]Prepared by reviewer.

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

***Significantly different from control value ($p \leq 0.001$).

TABLE 8. Non-neoplastic Liver Lesions in Mice Fed Chlordane Technical For Two Years[a]

Lesions	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	12.5	0	1	5	12.5
No. examined.	71	71	72	72	72	72	71	72
Hepatocellular swelling and degeneration	5	8	58***	59***	3	2	24***	59***
Fatty degeneration	2	0	3	9**	1	2	9	9
Necrosis	6	7	23***	19***	14	8	15	20

[a]Does not include animals sacrificed at 12 months.
**Significantly different from control value ($p \leq 0.01$).
***Significantly different from control value ($p \leq 0.001$).

TABLE 9. Frequently Occurring Non-Neoplastic Lesions In Mice Fed Chlordane for Two Years[a]

Organ/Lesion	Males/Dose (ppm) 0	1	5	12.5	Females/Dose (ppm) 0	1	5	12.5
Kidney	(71)[b]	(71)	(72)	(72)	(72)	(72)	(71)	(72)
glomerular nephrosis	38	33	42	46	23	34	35	48
glomerular amyloidosis	14	15	18	13	9	5	11	9
pelvic dilation	6	11	19	6	2	2	0	2
cyst formation	8	4	9	7	5	5	2	5
Heart	(71)	(71)	(72)	(72)	(72)	(72)	(71)	(72)
interstitial amyloid	21	19	11	10	9	7	15	15
Lung	(71)	(71)	(72)	1(72)	(72)	(72)	(71)	(72)
congestion	4	5	4	12	2	5	2	0
edema	3	8	3	7	1	3	0	1
amyloid deposits	15	17	10	9	6	7	14	14
Testis	(71)	(71)	(72)	(72)				
tubular necrosis	11	8	13	9				
decreased spermatogenesis	14	14	21	16				
Prostate	(69)	(69)	(71)	(71)				
prostatitis	0	5	0	0				
Seminal vesicle	(70)	(70)	(72)	(70)				
luminal dilation	14	25	26	19				
Urinary bladder	(70)	(70)	(72)	(72)	(72)	(72)	(71)	(72)
dilation	11	27	23	10	3	1	0	0
Ovary					(72)	(72)	(71)	(72)
cyst formation					27	23	23	35

[a]Does not include animals sacrificed at 12 months.

[b]The number in parenthesis is the number of tissues examined histologically.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The study authors identified the liver as the target organ. Technical chlordane was evaluated as tumorigenic since there was a significant increase in the incidence of hepatic adenomas and hemangiomas in males in the 12.5 ppm group when compared to controls. Tumors of the liver were not increased in dosed females and tumors in other organ sites in both sexes were not significantly different from controls. The incidence of tumors in the control groups in the study fell between the laboratory's spontaneous incidence for ICR-CRJ and ICR-JCL strains of mice.

Non-neoplastic changes in the liver were found in both sexes; there was hepatocyte swelling as early as 52 weeks of the study. There was an associated increase in liver weights for males that were sacrificed at 52 weeks in the groups receiving 1, 5, or 12.5 ppm chlordane, but no changes in dosed females. At the 24-month sacrifice, there was an increased incidence of hepatocellular swelling and necrosis in the 5 and 12.5 ppm groups of males and increased incidence of hepatocyte fatty degeneration in the 12.5 ppm group of males. Females in the 5 and 12.5 ppm groups had an increased incidence of hepatocyte swelling compared to control but other lesions of the liver occurred at the same incidence in all groups of females.

There were no compound/dose-related changes in signs of toxicity, body weight, or food consumption. Urinalysis and hematology did not reveal any compound-related changes. However, there was a "tendency towards elevated GOT AND GPT" in both dosed males and females. The NOEL was 1 ppm (0.123 mg/kg/day in males and 0.138 mg/kg/day in females). A "minimum toxic level" was 5 ppm in both sexes and a "certain toxic level" based on pathological examination and in-life measurements was 12.5 ppm, which corresponded to 1.645 mg/kg/day in males and 1.646 mg/kg/day in females.

A quality assurance statement, signed and dated December 1, 1983, was present.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We agree with the conclusions of the study author that chlordane is oncogenic in ICR mice, causing a significant increase in the incidence of hepatic adenomas and hemangiomas in males of the 12.5 ppm group. When the data for liver neoplasms in males was statistically analyzed using the test for linear trend in proportions, we found a significant positive trend for hepatocellular adenomas, hepatocellular adenocarcinomas and hemangiomas ($p < 0.05$). In tabulating and statistically analyzing the histopathology data, however, the report included animals sacrificed by design at 52 weeks. The tabulations in this review have censored findings for the interim sacrificed animals and presented the interim sacrifice

findings in the liver separately (Table 8). Statistical reanalysis of the data by our reviewers indicated that the censoring of the 12-month data did not change the level of significance of the histologic findings. The non-neoplastic changes in the liver occurred earlier in males than in females and at the 24-month sacrifice were more severe in males than in females. There was a correlation betwen hepatocyte swelling and liver weights in dosed animals. The report authors stated that there was a tendency for increases in GOT and GPT in chlordane dosed males and females and that there was a correlation between increases in these enzymes in blood of individual mice and hepatocellular swelling in these same mice (CBI p. 20). We do not feel that this conclusion is supported by the data. Data on the mean levels of these enzymes are tabulated in Table 4. There was a significant increase in SGPT only in males receiving 12.5 ppm at the terminal sacrifice and in females receiving 5.0 ppm at the 52 week sacrifice. However, there was quite a lot of variability in the data as indicated by the large standard deviations (Table 4). For control males at terminal sacrifice, the levels of GOT ranged from 53-1266 IU/liter, and 13 of 27 values were above 100 IU/liter (CBI Appendix 13-3), which is considerably above the normal range. The procedure for storing samples before analysis was not reported.

Although there were no significant increases in the incidence of any nonneoplastic lesion at any site other than the liver (using chi-square test), there was an apparent increase in renal glomerular nephrosis in both sexes receiving diets containing 12.5 ppm chlordane and amyloid deposition in the lungs of females at 12.5 ppm as compared to controls (Table 9). The incidence of systemic amyloidosis was fairly high in all groups but did not differ between groups.

The authors stated that there were no abnormalities in urinalysis. However, there was a high incidence of ketone bodies in all groups at 104 weeks; 16/27 and 17/26 in control and high-dose males and 19/30 and 29/34 in control and high dose-females, respectively.

The clinical studies were not performed at a sufficient number of intervals since hematology and urinalyses were not performed at 3, 6, or 18 months and biochemistry studies were not performed at the beginning of the study. Also, ophthalmologic examinations were not performed. The amounts of chlordane analyzed in the diets was considerably lower than the nominal concentrations and stability data of chlordane in the diet were not provided.

15. COMPLETION OF ONE-LINER FORM FOR STUDY: Not applicable.

16. CBI APPENDIX:

Appendix A, Analysis of Test Material, CBI Appendix 28.
Appendix B, Protocol, CBI pp. 5-12.