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

AUG 30 1989

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id# 52904-C. Lindane: Review of interim report of the rat chronic feeding/oncogenicity study with lindane (Life Sciences Research, #88/CIL002/816, 3/7/89) covering the 30 day and 26 week interim sacrifices. Confirmation that male rat kidney is a target organ for lindane toxicity. Request for quantitative data on alpha 2_u globulins in male kidney tissue.

TOX CHEM No.: 527
TOX PROJECT No.: 9-1545
Record No.: 246014
MRID # 410941-01 (three volumes)

FROM: John Doherty 8/28/89
Section I, Toxicology Branch I (IRS)
Health Effects Division (H5709C)

TO: George LaRocca
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THROUGH: Edwin Budd 8/28/89
Section Head
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The Centre International d'Etudes du Lindane (CIEL) through its counsel McKenna, Conner and Cuneo (refer to letter from Charles A. O'Connor, III dated May 3, 1989) has submitted an interim report on the rat chronic feeding/oncogenicity study with lindane. This study was especially designed to assess for the effects of lindane on the kidneys of this species as well as the other usual endpoints expected for a chronic feeding/oncogenicity study.

Toxicology Branch Comments

1. The interim report covering the 30 day and 26 week interim

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sacrifices was reviewed and the attached DER has been prepared. Refer to the DER for the summary of the toxicity responses noted thus far in the progression of the study. Certain aspects of the study are discussed as follows.

2. Kidney effects.

A. General.

The data thus far generated demonstrate that lindane affects the kidney of males. This observation is consistent with findings in the subchronic oral, dermal and inhalation rat studies with lindane. An effect in the male kidneys was evident by weight increases and structural changes indicated by histopathology (hyaline droplets in the proximal tubules, tubular regeneration, interstitial chronic nephritis and cortical tubular necrosis).

B. Possible effects on the functional capacity of the kidney.

The study report concluded that the functional capacity of the male kidney was affected as indicated by increased urine volume, water consumption, decreased specific gravity and indications of increased urinary protein.

In the opinion of this reviewer, the data presented do not provide a convincing case that lindane actually directly affected the functional capacity of the kidney.

Three different methods to assess urinalysis were done (routine, water loading and water deprivation), TB-I notes that there was little consistency among the three methods of analysis and for the three times the assessments were made for the supposed effects on urine volume, water consumption and specific gravity. Creatinine clearance, which is considered a reliable index of the functional capacity of the kidney, was not affected.

It is therefore difficult for TB-I to conclude that the functional capacity of the kidney was actually affected by lindane. In particular, urine volume, water consumption and decreased specific gravity are all interrelated and may be an anomaly unrelated to lindane effects. If structural damage of the kidney were sufficient to affect the functional capacity of the kidney, the expected findings would be decreased urine volume, and increased blood and protein in the urine and impairment of the creatine clearance. Of these only protein content may have been affected but not statistically significantly.

The possibility of impairment of the functional capacity of the kidney will be reevaluated in the subsequent interim (one year and 78 week) and final reports for this study. In these subsequent reports the registrant is requested to present in a single table all of the data which indicate that the functional capacity of the kidney is impaired. This table should show only those parameters believed to be affected by lindane (such as water consumption, urine volume, specific gravity, protein concentration and any others) and the data value for each dose level. The data for each parameter obtained from each method of urinalysis (routine, water deprivation and water loading) and at each assay time should be presented. A sample table as prepared by TB-I is attached.

C. Pathogenesis of the lindane induced lesions in the male kidney.

The observations noted thus far regarding the kidney are consistent with the possibility that lindane may be a chemical that specifically affects the male rat kidney and fits in the alpha 2_u globulin model of specific kidney toxicity.

In order to establish whether or not lindane fits the alpha 2_u globulin model of specific kidney toxicity, TB-I requests that the kidney tissue remaining from the 30 day and 26 week interim sacrifices and all kidney tissue from the subsequent sacrifice intervals be quantitatively analyzed for the presence of alpha 2_u globulins.

If the results of the analysis of the kidneys for alpha 2_u globulins together with other data allow the conclusion that lindane fits the alpha 2_u globulin model of specific kidney toxicity, the Agency will then determine if it is appropriate to regulate lindane on the basis of its effects in the male rat kidney or to use some other endpoint of toxicity.

D. Time course of the effects of lindane on the male kidney.

The absolute and relative weights of the kidneys in the group receiving 100 ppm were elevated at 30 days but not at 26 weeks. None of the rats were reported as having tubular regeneration at 26 weeks for the group dosed with 10 ppm but 9 of the 10 rats in this group had this condition at 30 days.

Only 2 of 10 rats (not significant) had cortical tubular necrosis in the group dosed with 100 ppm at 26 weeks but all 10 of 10 had this condition for this dose group at 30 days. For the group dosed with 400 ppm, 9 of 10 rats had the necrotic condition at 30 days but only 5 of 10 rats had this condition at 26 weeks.

Thus both the organ weight and histopathological data indicate that the effect of lindane on the male kidney is more

severe at 30 days than at 26 weeks.

The time course for the effects of lindane on the male kidney will be reevaluated when the additional interim and final reports of this study are submitted.

3. Deaths in Females.

There were 8 deaths in the high dose female group but only 2 in the control and 0 to 1 in the groups dosed with lower doses of lindane. No cause of death could be determined. This observation of females being more sensitive to lethal effects of lindane and a failure to determine the cause of death was also noted in both the rat subchronic dermal toxicity study (refer to J. Doherty review dated May 18, 1989) and the mouse subchronic inhalation study (refer to J. Doherty review dated June 30, 1989).

4. The pathology summary tables as well as other tables in this report were reproduced in a manner that resulted in very fine print that was blurred in many places and difficult to read. TB-I specifically requests that in future reports for this study the tables be prepared in a more readable print.

5. The interim report did not have a Quality Assurance Statement. The registrant is reminded that these statements must be included in the future reports.

Table. Summary of data indicating an effect of lindane on the functional capacity of the kidney. Data for males only.

Parameter		I[1]	Control	1 ppm	10 ppm	100 ppm	400 ppm
Water Consumption (ml/rat/day)		30d	44	44	41	55	52(H)
		26w	50	38	43	42	63(H)
Urine Volume (ml)	(R)	3/4w	5.5	6.0	7.0	7.5*	7.5*
	(WL)	3w	11.0	10.0	9.0	11.5	11.0
	(WD)	3w	5.5	5.5	6.0	8.0*	7.5
	(R)	12/13w	5.0	4.5	5.5	6.0	7.5***
	(WL)	12w	14.0	15.5	13.0	14.0	14.5
	(WD)	12w	4.0	4.5*	7.5	6.5	8.0**
	(R)	24/25w	9.0	9.5	8.5	9.5	11.5
	(WL)	24w	15.0	14.0	16.5	14.5	15.5
	(WD)	24w	7.5	5.0*	8.5	7.5	9.5
Specific Gravity (gm/ml x 1000)	(R)	3/4w	1053	1049	1045*	1040*	1043**
	(WL)	3w	1016	1016	1017	1014	1018
	(WD)	3w	1041	1047	1041	1034	1035
	(R)	12/13w	1055	1061	1050	1045**	1043***
	(WL)	12	1016	1016	1017	1014	1018
	(WD)	12	1052	1052	1042*	1044	1037**
	(R)	24/25w	1032	1032	1034	1035	1032
	(WL)	24	1011	1012	1011	1011	1012
	(WD)	24	1035	1045**	1037	1040	1034
Protein Concentration (mg%)	(R)	3w	107	120	129	107	107
	(WL)	3w	36	60	50	48	120(H)
	(WD)	3w	240	240	340	300	480(H)
	(R)	12w	133	125	99	139	173(H)
	(WL)	12w	20	30	20	20	68(H)
	(WD)	12w	146	170	84	150	220(H)
	(R)	24w	64	87	81	40	102(H)
	(WL)	24w	20	16	20	26	38
	(WD)	24w	142	230	98	128	180

* P < 0.05, ** P < 0.01, *** P < 0.001
(H) Statistical evaluation not made, H indicates the quantitive value was much greater than the control.
R= routine analysis, WL= water loading, WD= water deprivation.
[1]I= Time of test, d= days, w= weeks.

Reviewed By: John Doherty 8/22/89
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Edwin Budd 8/23/89
Section I, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 83-1 and 83-2 - Chronic Feeding and Oncogenicity (30 day and 26-Week Interim Report)

MRID No.: 410941-01 (3 volumes) TOX CHEM No.: 527

Test Material: Technical Lindane (Batch DA 433)

Synonyms: Gamma-hexachlorocyclohexane

Test Animals: Rats - Wistar strain obtained from the Charles River Breeding Laboratories, Kingston, New York. Housed 5 per cage.

Study Number(s): 88/CIL002/816

Sponsor: Centre Internationale de Etudes du Lindane (C.I.E.L.)

Testing Facility: Life Science Research Limited, Eye, Suffolk, England

Title of Report: "Lindane: Combined Oncogenicity and Toxicity Study by Dietary Administration to Wistar Rats for 104 Weeks - Interim Report Week 0-26" (929 pages in 3 volumes).

Author(s): S.J. Amyes

Report Issued: March 7, 1989

Conclusions:

This information is SUPPLEMENTARY since it is an interim report (up to 26 weeks) of an ongoing chronic feeding/oncogenicity study. The following NOEL and LELs have been indicated by the data generated thus far.

NOEL = 1 ppm

LEL = 10 ppm. At this level (and above) there are effects in the male kidney characterized by hyaline droplets in the proximal tubules and tubular regeneration.

At 100 ppm (and above) there are additional effects noted in the kidney of males described as interstitial chronic nephritis, cortical tubular necrosis, and kidney weight increase. The liver of both sexes have periacinar hypertrophy (only males were statistically significant).

At 400 ppm deaths in females (unknown cause); increases in liver weight (both sexes); early (transient) decreases in body weight gain. Possible impairment of the functional capacity of kidney (males) as indicated by increased water consumption and urine volume, and decrease in specific gravity of urine and slightly higher protein readings (study report conclusion).

Classification: SUPPLEMENTARY (Interim Report)

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement:

No Quality Assurance statement was found with this submission.

Review

The basic design of this ongoing study consists of 115 male and 115 female rats per group which are being dosed with either 0, 1, 10, 100, or 400 ppm of lindane in the diet for up to 2 years. The breakdown of the interim sacrifice groups is as follows (each interim sacrifice group consists of 15 males and 15 females, the terminal [oncogenicity] group consists of 55 males and 55 females):

30-day sacrifice;
26-week sacrifice;
52-week sacrifice;
78-week sacrifice (These rats are scheduled to be dosed with lindane for 52 weeks and allowed to live for 26 additional weeks to assess for reversibility of any lindane effects); and
104-week sacrifice (oncogenicity aspect of study).

Additional groups of 10 male and 10 female rats were designated as "veterinary controls" and maintained on control diets to investigate any possible disease outbreaks in this study.

The test diets were prepared by mixing lindane into the feed (LAD-2, supplied by Tabrune Manea, Cambridgeshire, England) and diluting a premix with feed to obtain the desired concentrations. Tests diets were prepared weekly and stored in the animal's room in light proof plastic storage bins. Tests for homogeneity, stability, and achieved dietary concentration were conducted. The homogeneity and stability tests were done prior to starting the study. The checks on achieved dietary concentration were run at weeks 1, 2, 3, 4, 13, and 26 of treatment. The analyses revealed that there was 113 $\pm$ 25, 99 $\pm$ 10, 97 $\pm$ 9, and 96 $\pm$ 3 percent of the desired concentration for the 1, 10 100, and 400 ppm test dose groups, respectively. The estimated shelf life for the 400 ppm diet was determined to be 22 days and that for the 1 ppm diet to be 16 days (stability tests for 10 and 100 ppm were not reported). Since the diets were prepared fresh weekly, they were used within their shelf life. Homogeneity was found to have a coefficient of variation of 10 and 2 percent for the 1 and 400 ppm mixtures, respectively.

Results

1. Clinical Signs and Reactions - There were no reactions to treatment reported. No dose-related increases in palpable swellings were evident.

2. Mortality - Seven males and 12 females died. Among the males, there were 1, 2, 2, 2, and 0 deaths for the control,

low, mid-1, mid-2, and high dose groups without any indication of a dose or treatment-related effect.

Among the females there were 2, 0, 1, 1, and 8 deaths indicating that the high-dose group had a higher rate of deaths (8) than control or lower dose groups. It should be noted that no symptoms were observed in the decedents which died during weeks 2 to 4. The cause of death could not determined.

NOEL (mortality) = 100 ppm. LEL = 400 ppm. Deaths in females.

3. Body Weight Gain, Food and Water Consumption - The mean body weight for the high-dose group males was about -6 percent less than the control for weeks 1 to 5 and gradually increased such that at week 26 the body weights were only about 2 percent different. The high-dose group females also had decreases in body weight gain for week 1 (about 8 percent) but the difference was only about -2 percent at week 9-10. The other groups were not similarly affected and had weight gains similar to the controls.

   NOEL (body weight gain) = 100 ppm. LEL = 400 ppm. Early and transient body weight decreases.

   Food Consumption was reported as being "marginally lower" in males and females receiving 400 ppm. Water consumption was reported as being "marginally higher" in males receiving 400 ppm. For example, the high-dose group consumed 63 mL/kg/day and the controls only 50 mL/kg/day, while the other groups consumed 38, 43, and 42 mL/kg day. Refer to section 7 (Urinalysis) below for table illustrating the water intake. Water consumption in females was reported as being similar in all dose groups.

4. Ophthalmoscopy - Assessments were made at weeks 3 and 24 using a Fissons binocular indirect ophthalmoscope after instillation of 0.5 percent tropicanimide into the rats eyes. The pretest ophthalmoscopy examination resulted in rejecting 16 males and 11 females.

   No treatment-related effects were evident at weeks 3 and 24.

5. Hematology - Samples of blood were taken from the retro-orbital sinus at pretest, at week 3 (from the 30-day sacrifice group) and at weeks 12 and 24 (from the 26-week sacrifice group). The samples were taken following overnight deprivation of food while the rats were held under light ether anesthesia and EDTA was used as an anticoagulant. The following parameters were investigated: Packed

cell volume (PCV), hemoglobin concentration (Hb), erythrocyte count (RBC), leukocyte count (WBC, total and differential), platelet count, mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), and mean cell hemoglobin (MCH).

Of these parameters, the following possible effects of lindane were noted:

a. Hb - The high-dose group females were decreased -6.4 percent ($p < 0.1$) at 3 weeks and -4 percent ($p < 0.5$) at 12 weeks and -7 percent ($p < .01$) at 24 weeks. The males were decreased -3 percent at week 12 only.

b. RBC - The high-dose group females were decreased -6 percent ($p < .001$) at week 3, and -6.3 percent at week 24.

c. PCV - The high-dose group females were decreased -4.2 percent ($p < .01$) at week 3, and -9.1 percent at week 24.

[The report states that males were "marginally lower" for these parameters but statistical significance was not attained.]

d. Platelet Counts - Platelet counts were generally higher for the dosed groups (both males and females). Statistical significance was attained especially at week 12 for males for the mid- and high-dose groups and week 24 for females (mid- and high-dose groups). The increases reached about 13 to 14 percent.

e. WBC (Total) - WBC counts were increased 27.5 percent (mid dose, $p < .05$) and 23.5 percent (high dose, $p < .05$) female groups. This corresponded with an increase in neutrophils for the mid +83.3% ($p < .09$) and high +83.3% ($p < .05$) dose groups.

CONCLUSION (Hematology) - The changes in blood parameters are not considered to be conclusively related to lindane at this time. The possible effects of lindane on these parameters will be reevaluated pending receipt and review of the subsequent interim and final reports for this study.

6. Clinical Chemistry - Blood samples taken for hematology (see above) were also analyzed for clinical chemistry parameters. The following were assessed for: alkaline phosphatase activity (AP), alanine aminotransferase activity (ALT), aspartate aminotransferase activity (AST), lactate dehydrogenase activity (LDH), gamma-glutamyl transpeptidase (GGT),

urea, creatinine, glucose, total bilirubin, total cholesterol, total protein, albumin, Na^+, Cl^-, Ca^{++} and phosphorus.

The following possible changes were noted:

a. Phosphorus and Ca^{++} - The following table indicates the percentage increases in these elements.

		Phosphorus		Calcium			
Group		M-2	H	L	M-1	M-2	H
3 Weeks	M	-	+7.3*	-	-	-	-
	F	-	+18.2***	+8***	+8***	+6***	+10***
12 Weeks	M	-	+8.1**	-	-	-	+3.6**
	F	-	+29***	-	-	-	+3.5**
24 Weeks	M	+6.9%*	+13.8***	+3.7*	-	-	+3.7**
	F	-	-	+3.6*	-	-	+3.6*

* $p < .05$, ** $p < .01$, *** $p < .001$

b. Urea - The high dose group females were elevated up to 53 percent (week 3) but by the 24th week this group was only 20 percent elevated. The males also showed increases especially at week 12 but a dose response was not evident and the maximum increase was in the 10 ppm group (+37.9 percent).

c. Total Cholesterol - The high-dose group females were elevated 40 and 53 percent at weeks 3 and 12 but an apparent increase at 24 weeks was not statistically significant. The high dose male group was elevated 29 percent at week 12 only.

ALT and AST activities were reported as being lower.

CONCLUSION (Clinical Chemistry) - There are some indications of possible effects of lindane treatment on phosphorus, calcium, urea, and cholesterol but at this time there is insufficient evidence to conclusively associate an effect of lindane. A clinical chemistry NOEL will be reevaluated pending receipt and review of the subsequent interim and final reports on this study.

Note: An increase in serum PO_4 is an indication of possible renal failure but the females rather than the males had the highest increases. Thus, it is doubtful that the increase

in phosphorus here is an indication of a renal effect.

7. Urinalysis - (Note: Because the kidney is regarded as a target organ for lindane toxicity, urinalysis in given special assessment). Three different types of urinalysis procedures were used as follows:

   a. Routine Analysis - Urine samples were collected while the rats were fasted overnight following previous exposure to food and water ad libitum for 48 hours (note prior to the 48-hour period, these rats were used for the procedures in b and c below).

   b. Water Loading - The rats were dosed with 20 mL/kg by gavage and urine was collected for the next 8 hours. It was not stated if the rats were deprived of food and water prior to water loading or if they were allowed any food or water during collection.

   c. Water Deprivation - The rats were deprived of food and water for 4 hours and their urine removed by supra-pubic pressure. This urine was discarded. The rats were furtner deprived of food and water overnight and during this time their urine was collected and latter analyzed.

   The urine from each procedure was analyzed for: appearance, volume, pH, specific gravity, protein, total reducing substance, glucose, ketones, bilirubin, urobilin, nitrite, blood, urea, creatinine and micropsy (of sediment).

   The following indications of possible effects of lindane were noted at either week 3(4), 12, or 24.

   The effects in females involved possible increase in specific gravity for the high-dose group at weeks 12 (1053 vs. 1043) and 24 (1050 vs. 1039) as indicated by routine analysis and by water deprivation at week 24 only (1043 vs. 1033). At week 24 the mid-1 group (10 ppm) was also elevated (1055 vs. 1039). Urea (+79%) and creatinine (+37.9%) were higher for females for the high dose group only when assessed by routine analysis. These observations in females are considered random and not related to treatment at this time (to be reevaluated in future interim and the final report for this study.

   The following effects were evident in males (refer to the Table on the following page):

1) Volume - Increased for males in the high-dose (and occasionally the mid-dose) groups as indicated by routine analysis and water deprivation, reaching increases of 36 to 100 percent. This reviewer does not consider that there was consistency over time for this increase.

2) Specific Gravity - The specific gravity was lower (i.e., 1043 vs. 1055) at week 12/13 for the high-dose group and on other occasions similar decreases were evident. Note: Possible increases were noted in females.

3) Protein Concentration - Elevations in protein concentration were reported but the data were not presented in the summary tables. TB-I made independent tabulations refer to Table next page.

4) Urea and Creatinine - Similar to the findings in females, these parameters were noted to be higher. For example, at week 12/13 the high-dose group was 22 percent higher for creatinine and at week 24 it was 20 percent higher at routine analysis. Following water deprivation, the creatinine was lower (-43 percent). TB-I does not consider that changes in creatinine demonstrated sufficient consistency to conclude an effect of lindane treatment.

   Urea values were elevated (as assessed by routine analyses) for the high dose group (+25 percent) at week 24/25 and 38 percent at week 12/13.

5) pH - The pH appeared to be lower (about 0.4 units) for the mid-2 and high dose groups. (Not shown in Table).

6) Micropsy - The number of epithelial cells was reported as being higher and number of crystals was lower but these data were not presented in the summary tables.

   Note: There was not always consistency between the three methods for assessing urinalysis. The only effects indicated by the water loading procedure, which is presumably a more reliable method, were protein concentration and specific gravity changes neither of which was affected to the extent that an effect of lindane on kidney function could be convincingly established at this time.

Creatinine and Urea Clearance - The creatinine and urea

Table. Summary of data indicating an effect of lindane on the functional capacity of the kidney. Data for males only.

Parameter		T[1]	Control	1 ppm	10 ppm	100 ppm	400 ppm
Water Consumption (ml/rat/day)		30d	44	44	41	55	52(H)
		26w	50	38	43	42	63(H)
Urine Volume (ml)	(R)	3/4w	5.5	6.0	7.0	7.5*	7.5*
	(WL)	3w	11.0	10.0	9.0	11.5	11.0
	(WD)	3w	5.5	5.5	6.0	8.0*	7.5
	(R)	12/13w	5.0	4.5	5.5	6.0	7.5***
	(WL)	12w	14.0	15.5	13.0	14.0	14.5
	(WD)	12w	4.0	4.5*	7.5	6.5	8.0**
	(R)	24/25w	9.0	9.5	8.5	9.5	11.5
	(WL)	24w	15.0	14.0	16.5	14.5	15.5
	(WD)	24w	7.5	5.0*	8.5	7.5	9.5
Specific Gravity (gm/ml x 1000)	(R)	3/4w	1053	1049	1045*	1040*	1043**
	(WL)	3w	1016	1016	1017	1014	1018
	(WD)	3w	1041	1047	1041	1034	1035
	(R)	12/13w	1055	1061	1050	1045**	1043***
	(WL)	12	1016	1016	1017	1014	1018
	(WD)	12	1052	1052	1042*	1044	1037**
	(R)	24/25w	1032	1032	1034	1035	1032
	(WL)	24	1011	1012	1011	1011	1012
	(WD)	24	1035	1045**	1037	1040	1034
Protein Concentration (mg%)	(R)	3w	107	120	129	107	107
	(WL)	3w	36	60	50	48	120(H)
	(WD)	3w	240	240	340	300	480(H)
	(R)	12w	133	125	99	139	173(H)
	(WL)	12w	20	30	20	20	68(H)
	(WD)	12w	146	170	84	150	220(H)
	(R)	24w	64	87	81	40	102(H)
	(WL)	24w	20	16	20	26	38
	(WD)	24w	142	230	98	128	180

* P < 0.05, ** P < 0.01, *** P < 0.001

(H) Statistical evaluation not made, H indicates the quantitive value was much greater than the control.

R= routine analysis, WL= water loading, WD= water deprivation.

[1]T= Time of test, d= days, w= weeks.

clearance was calculated after 3, 12, and 25 weeks based on plasma levels of endogenous creatinine and urea and creatinine and urea in the urine, urine volume and period of collection.

No consistent effect to indicate a dose related treatment response to lindane was evident.

CONCLUSION (Urinalysis): The magnitude and consistency of the changes in the urinary parameters reported do not provide a basis to conclude that lindane is affecting the functional capacity of the kidney. The study report asserts that urinalysis parameters are affected at the 400 ppm dose level.

8. Organ Weights - The following organs were weighed at the 30-day and 26-week sacrifice intervals: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, thyroid (and parathyroids).

Of these organs, there were possible increases in the kidney and liver as indicated in the following table.

Organ	Sex	I[2]	Dose Group[1/] 1 ppm	10 ppm	100 ppm	400 ppm
Kidney						
Absolute	M	30d	-	-	+16.9**	+12.9%**
	M	26w	-	-	-	+39.3%**
Relative	M	30d	-	-	+23.6**	+27.3%**
	M	26w	-	-	-	+34.0%**
Liver						
Absolute	M	30d	-	-	-	-
	M	26w	-	-	-	+40.8%**
Relative	M	30d	-	-	-	+14.0*
	M	26w	-	-	-	+34.7**
Absolute	F	30d	-	-	-	+29.2**
	F	26w	-	-	-	+32.3%**
Relative	F	30d	-	-	-	+37.2**
	F	26w	-	-	-	+31.7**

1/Data are in % relative to control.
2/I = 30 day of 26 week interval
-/Data are equivalent to control.
*/p < .05, **/p < .01.

Other organs showing occasional statistically significant weight differences are listed as follows:

a. Adrenals - high-dose group males at week 26 were increased 34 percent absolute, 28 percent relative.

b. Brain - On day 30, the high-dose group males were increased 15.2 percent (relative weight only).

- At week 26, the high dose group females were 7 percent increased.

c. Spleen - On day 30, the mid- (+14.6) but not the high-dose group males were elevated. The high dose group females (+21.5%) was also elevated.

- At week 26, the high dose group females were increased (+16.7%). All increases were in relative weight only.

d. Thymus - On day 30, the high-dose group females were increased (+23%).

CONCLUSION (Organ Weights) - NOEL = 10 ppm. LEL = 100 ppm. Kidney weight increases in males (early or at 30 days). At 400 ppm, further increases in kidney weight (males) and increases in liver weight (both sexes).

The weight changes in adrenals, brain, spleen and thymus are not regarded at this time as being compound related. These organs will be reevaluated for weight changes in the later interim and final reports for this study.

9. Bone Marrow Analyses - Two bone marrow smears from the femur and sternum were taken from each rat and prepared for analysis including staining with Pappenheimer and Wright stains.

No intergroup differences in cellularity and/or composition of the cells was noted . It is noted, however, that the individual cell types were not quantitated and Appendix 19 reports only statements that each sample is normal or comments on the quality of staining.

The manner of presentation of the bone marrow analyses is inadequate. Consideration should be given in future reports

to presenting the bone marrow analyses in a more meaningful manner.

10. Macroscopic Observations - The only lesions noted at necropsy were increased incidence of "pale kidneys" in the males at 100 and 400 ppm. Refer to Histopathology discussion below.

11. Histopathology - A comprehensive list of some 42 tissues/organs were prepared and examined microscopically for the control and high-dose group or from those rats which died or were sacrificed moribund. Only the testes, spleen, liver, bone marrow (sternum and femur) and gross lesions were scheduled to be examined for the low and mid dose groups.

Of these organs only the liver (both sexes) and kidney (males only) were noted to have indications of lindane-related change. The following topical organ discussions illustrate these findings.

a. Kidney - The kidney of males is regarded as a target organ for lindane toxicity. In this study kidney weight changes and increases in "pale kidneys" in males were evident. The study report also asserted that kidney function in males was affected. Six transverse sections from each rat were prepared, three from each kidney. Both H&E stain and a special staining with Masson's Trichrome was used. The following table illustrates the histopathological findings in the kidney.

		Dose Group				
Lesion	N[1]	C	1 ppm	10 ppm	100 ppm	400 ppm
		10/9	10/10	10/10	10/10	10/10
Hyaline Droplets in Proximal Tubules[2]	30d[3]	0	0	10***	10***	10***
	26w	0	4	10***	10***	10***
Tubular Regeneration	30d	0	0	9***	10***	9***
	26w	0	0	0	8***	7**
Interstitial Chronic Nephritis	30d	0	0	0	8***	6*
	26w	0	0	0	6*	5*
Cortical Tubular Necrosis	30d	0	0	0	10***	9***
	26w	0	0	0	2	5*

$p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

1/Number examined at 30 days/number examined at 26 weeks.
2/As indicated by Masson's Trichrome (only nine in the 10 ppm group were indicated by H/E stain).
3/30 day and 26 week analyses.

The above table suggests that the kidney is more severely affected at 30 days than at 26 weeks based on the incidence of tubular regeneration, interstitial chronic nephritis and cortical tubular necrosis.

There were no indications of dose-related increases in these lesion types in the female. For example, at week 26 there were no incidences of these lesion types in females.

b. Liver - Liver weight was increased and pathological changes in the liver were evident in other studies with lindane (subchronic).

			Dose Group				
			0	1 ppm	10 ppm	100 ppm	400 ppm
Lesion		I[1]	N (10/8)	(10/10)	(10/10)	(10/10)	(10/9)
Periacinar Hepatocytic Hypertrophy	30d[2]	M	0	0	0	7**	10***
		F	0	0	0	2	9***
		N	(9/10)	(10/10)	(10/10)	(10/10)	(10/9)
	26w	M	0	0	2	8**	10***
		F	0	0	0	2	9***

** $p < .01$, *** $p < 0.001$
1/ number of males/number of females examined
2/ 30 day or 26 week analyses

c. Other Topical Organ Discussion:

- Adrenals, brain, spleen, and thymus weights were increased but there were no dose-related pathological changes in these organs.

CONCLUSION (Histopathology) - NOEL = 1 ppm. LEL = 10 ppm. Hyaline droplets in the proximal tubules and tubular regeneration in kidneys, LEL = 100 ppm; interstitial chronic nephritis and cortical tubular necrosis in male kidney, also periacinar hypertrophy in liver of both sexes but only the males were statistically significant at 100 ppm. Females were statistically significant at 400 ppm.

12. Proof of Absorption - Blood samples were taken from the sacrifices made at 30 days and 26 weeks. These samples have either not yet been analyzed or their results have not been prepared into a report.

CONCLUSIONS - This information is SUPPLEMENTARY since it is an interim report (up to 26 weeks) of an ongoing chronic feeding/ oncogenicity study. The following NOEL and LEL have been indicated by the data generated thus far.

NOEL = 1 ppm

LEL = 10 ppm. At this level (and above) there are effects in the male kidney characterized by hyaline droplets in the proximal tubules and tubular regeneration.

At 100 ppm (and above) there are additional effects noted in the kidney of males described as interstitial chronic nephritis and cortical tubular necrosis and kidney weight increase. The liver of both sexes has periacinar hypertrophy (only males are statistically significant).

At 400 ppm deaths in females (unknown cause); increases in liver weight (both sexes); early (transient) decreases in body weight gain. Impairment of the functional capacity of kidney (males) as indicated by increased water consumption and urine volume, and decrease in specific gravity of urine and higher protein readings (study report conclusion).

Note: The following parameters not mentioned above in the LEL determinations should be reexamined in the future interim and final sacrifice reports because these showed possible effects of lindane in this report:

1. Hb, RBC, PCV, platelet counts and WBC count at hematology.

2. Phosphorous, calcium, urea and cholesterol in serum.

3. Adrenal, brain, spleen and thymus weights (and any associated histopathology).