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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id. No.: 52904-C. Lindane: Review of a 13-week dermal toxicity study with rats.

TOX CHEM No.: 527
TOX PROJECT No.: 8-1219
Record No.: 231696

FROM: John Doherty 5/17/89
Section I, Toxicology Branch I (IRS)
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TO: George LaRocca
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THROUGH: Edwin Budd
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Budd 5/17/89

The Centre International d'Etudes Du Lindane (C.I.E.L.) has submitted a 13-week dermal toxicity study with lindane in rats in response to a previous request from Toxicology Branch (refer to Addendum to Lindane Registration Standard. Requirement for Additional Toxicity Studies, memo from E.R. Budd dated December 5, 1985). The purpose of this study was to further assess the toxicity of lindane especially regarding the potential to affect the kidney resulting from dermal exposure. The study also included special analysis of the bone marrow to further evaluate the possibility of lindane affecting the formed elements of the blood.

The study was reviewed by Toxicology Branch I (TB-I) and the following comments apply.

Toxicology Branch Comments

1. This study provides further evidence that the male rat kidney

develops histopathological changes (in this case increased intensity of hyaline droplet formation, tubular degeneration with necrosis, basophilic tubules both regenerative and atrophic, and casts) in response to lindane exposure. Male kidney weight was also increased.

Female kidney data did not indicate lindane-related increases in tubular degeneration, casts or hyaline droplets (lesions that were dose related in the males) and kidney weight gain was not affected. At worst, the incidence of basophilic tubules (regenerative) was possibly higher in the high dose test group at each sacrifice interval. Overall, TB-I does not consider that the female kidneys were affected in this study.

2. A definite NOEL for histopathological changes in the male rat kidney was not established by the data presented. At the lowest dose tested (10 mg/kg/day) there was evidence of increased intensity of hyaline droplet formation, tubular degeneration with necrosis, basophilic tubules both atrophic and regenerative and casts.

Since these effects were only slight at 10 mg/kg/day, TB-I does not consider it necessary to require an additional subchronic dermal study to establish a more definite NOEL.

3. Evidence of tubular degeneration with necrosis persisted after the six week recovery period to indicate that the histopathological changes in the male kidney were not fully reversible. The intensity of hyaline droplet formation and kidney weight, however, were equivalent to the controls after the recovery period indicating that some aspects of the kidney pathology are more readily reversible.

4. The evidence for impairment of the functional capacity of the male kidney was <u>indefinite</u> as indicated by there being some apparent evidence of increases in urinary protein, turbidity and blood scores and casts. These increases did not demonstrate clear dose responses. The increases in urinalysis parameters were more apparent at week 7 but less apparent at week 14 when the histopathological changes in the kidney were most evident. <u>Lastly, there were no changes in the creatinine clearance which is considered a more reliable estimate of the functional capacity of the kidney</u>.

In conclusion, TB-I considers it equivocal whether or not lindane treatment resulted in actual changes in urinary parameters. Resolution of this issue would require <u>quantitative</u> analysis of the urine rather than the semi-quantitative methods used which employ +,++, +++ etc as an index of the concentration of the blood, protein and turbidity.

Resolution of the issue of lindane causing changes in blood,

protein and turbidity scores in the urine is not considered essential by TB-I because the histopathological changes noted at the lowest test dose level are considered sufficient for a regulatory endpoint.

5. There was no evidence of any effects of lindane treatment on the formed elements of the blood as indicated by bone marrow analysis.

6. The study report (page 39, Volume 1) states that "the kidney of the male rat is clearly unusual in its ability to accumulate materials such as Lindane. It may therefore be inappropriate to establish the male rat kidney alone to establish a NOEL for extrapolation to man".

TB-I recognizes that the male rat kidney may be unique in its response to certain xenobiotics and it is possible that in the future certain effects on the male rat kidney may be considered irrelevant to human health hazard assessment. For example, if the effects of lindane can be demonstrated to fit into the alpha 2_u globulin model, a model that is being studied as a specific response in the male rat kidney, than a case may be made for not regulating lindane on the basis of its kidney effects.

TB-I has previously made a request to C.I.E.L to assess the kidney tissue remaining from this 13 week dermal toxicity study and for the ongoing rat chronic feeding study for alpha 2_u globulin content (refer to Doherty memo dated February, 1989).

<u>In this regard, the subchronic dermal toxicity study reviewed here (Hazleton, #5757-580/2, issued August 1988), is classified as SUPPLEMENTARY pending receipt and review of the requested information regarding alpha 2_u globulins. The study will be reconsidered for CORE classification when this information is submitted.</u>

Reviewed By: John Doherty 4/13/89
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Edwin Budd
Section I, Toxicology Branch I - IRS (H7509C)

EB 4/13/89

DATA EVALUATION REPORT

Study Type: 82-3 - Subchronic Dermal - Rat

MRID No.: 408217-01 (4 Volumes) TOX Chem No.: 527

Test Material: Lindane (Batch No. 433); > 99.5% Purity

Synonyms: gamma-hexachlorocyclohexane

Study Number: 5757-580/2

Sponsor: Centre International d'Etudes du Lindane

Testing Facility: Hazleton, UK (North Yorkshire, England)

Title of Report: Lindane: 13-Week Dermal Toxicity Study (With Interim Kill and Recovery Period) in the Rat.

Author: D. Brown, B.Sc., Ph.D.

Report Issued: August 1988 [No specific study report date]

Conclusions:

NOEL < 10 mg/kg/day. At this dose level and above pathological changes in male kidney structure are evident (increased intensity of hyaline droplets, tubular degeneration with necrosis, basophilic tubules both regenerative and atrophic and casts).

At 60 mg/kg/day and/or above, increased evidence of male kidney pathological changes, liver (both sexes) and kidney (males only) weight increases; centrilobular hypertrophy of the liver; serum cholesterol; behavioral changes (minor); body weight increases (both sexes). Possible increased deaths in high dose female group.

Urinalysis data were considered equivocal with regard to assessing a functional impairment of the kidney.

Doses tested: 0, 10, 60 and 400 mg/kg/day.

Classification: SUPPLEMENTARY pending receipt and review of additional information concerning quantitation of alpha 2_u globulins.

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

Quality Assurance Statement:

A statement signed by Pamela R. Cooper, Quality Assurance Manager attested that the study was audited/inspected by the independent HUK Quality Assurance Unit a total of 15 times. Eleven reports were prepared from these audit/inspections.

Basic Study Design:

The basic design of this study consisted of dosing four groups of 49 male and 49 female rats (Crl:(WI)BR strain obtained from the Charles River UK, Ltd. Margate, England) dermally with lindane at the dose levels of 0, 10, 60, or 400 mg/kg/day. Groups of 13 of each sex were sacrificed after 6 weeks of treatment. The main phase of the study consisted of 23 of each sex per group and was treated for 13 weeks before sacrifice. A third (recovery phase) group consisting of 13 of each sex was retained in the study for an additional 6 weeks without treatment after the 13-week treatment period.

The dose levels selected for this study were based on a preliminary range-finding study (Hazleton, UK Report #5577-580/5).

The test rats were 5-7 weeks old on arrival. They were acclimatized for 17 days before initiation on the treatment program when they weighted 257-343 g (males) and 161-236 g (females). They were housed individually.

Lindane was suspended in 5% aqueous carboxymethylcellulose and applied at a constant dosage volume of 4 ml/kg to the clipped area of the skin on the dorsal surface of the rats. The rats were reported as being clipped at weekly intervals. The test material was retained at the site of application by means of a specially designed dressing of porous gauze heat-welded to aluminum foil which was in turn retained by an elastic self-adhesive bandage. The rats were fitted with plastic collars to prevent the ingestion of the test article. Treatment with lindane (or 5% aqueous carboxymethylcellulose) was for 6 hours per day, on 5 consecutive days per week. The rats were not treated on the day of necropsy.

The rats were sacrificed at their scheduled time by intraperitoneal injection of sodium pentobarbitone and exsanguination by cutting the dorsal aorta and vena cava following an overnight period without food.

Methods and Results:

A. Analysis of the Test Formulation, its Stability and Other Factors Related to Test Material Application
[Refer to Appendix I of the study (pages 112 to 130) for the report prepared by K.G. Curl]

During the first week, the analysis of the test material indicated that the concentration of lindane was

below (as much as 60% for the high-dose group) the target level. This decrease was eventually determined to be attributable to the settling of lindane in the carboxymethylcellulose solution. Subsequently it was shown that the homogeneity of the lindane mixture was stable for 60 minutes when the samples were stirred mechanically. The achieved concentrations of later lindane solutions were close to 100 percent of the target levels.

B. Mortality and Clinical Behavior Reactions to Treatment

Among the males, there was no indication of treatment-related mortality. There were 34 to 36 out of 36 males in each group which survived the entire dosing period. [A total of two original male rats were removed and replaced with new rats in the first 2 weeks because of apparent poor appearance, one in the control and one in the mid-dose group.] The cause of the 1 to 2 deaths per dose group was not determined.

Among the females, a total of 16 rats were replaced during the first 2 weeks of the study: two in the control group; one in the low-dose group; three in the mid-dose group; and 10 in the high-dose group. There were 31, 30, 33, and 26 female original rats which survived to week 13 (the entire dosing period). The study report indicates that the high apparent rate of deaths in the high-dose female group (a difference of only 5 rats) was "suggesting a possible response to treatment." The cause of death among the females [redacted] rats was not indicated.

The behavioral signs included:

"Aggressiveness or hyperactive" - Both male and female treated rats had higher incidence than the controls, but a dose response was apparent only in females during week 1 and again in weeks 5 to 9, and later at weeks 11 to 13. At most about 1/3 of the females in the high-dose group had this symptom.

"Languid" - This symptom was most apparent in mid- and high-dose females during weeks 1 to 9. During the first week, 19 females in the high dose group and none in the control were affected but no more than 7 per group were affected thereafter.

"Piloerection, salivation, hunched" - 22 females in the high dose group and none in the control group were affected during the first week. During weeks

5 to 6 as many as seven in the high-dose group were affected.

Other symptoms such as "rapid respiration and wheezing," ataxia and tremors convulsions and "tense" were evident in four or less females in the high-dose group and only occasionally in the other groups.

Among the males, too few animals were affected with any of the symptoms to determine with certainty that lindane produced behavioral reactions in this sex. Although a few of the treated male rats were reported to show aggressiveness and hyperactivity, no consistency or dose response was evident.

Among the females, a NOEL of 10 mg/kg/day and LEL of 60 mg/kg/day is assigned for behavioral reactions. At the LEL and higher, there are signs of compound-related piloerection, salivation, hunched (appearance) and languid appearance. At 400 mg/kg/day "aggressiveness and hyperactivity" were also observed. Similar behavioral reactions were not observed during the recovery period. There was also a possible increase in the number of deaths among the females dosed with 400 mg/kg/day.

The local (dermal) reactions to treatment included "sores/lesions, desquamation or redness on treated area." It could not be established if the rats dosed with lindane actually were more severely affected than the control rats. For example, at some weekly intervals, there were more lindane-treated rats affected than the control groups, but on other weeks, more or as many control rats were affected.

C. Body Weight Gain and Food Consumption - It was reported that decreases in body weight gain of 2 percent for males and 4 percent for females were evident in the high-dose groups during the first week of the study. At the end of 6 weeks, the male body weights in the high-dose group were 4 to 5 percent less than the controls.

Overall, however, body weight increases were an apparent effect of lindane treatment as indicated in the following tables copied from the study report.

Mean Body Weight Gain (g) and Standard Deviation

Mean Gain Over Weeks		Group and Sex 1M	2M	3M	4M
0-6	Mean	91.7	84.2	86.9	78.8**
	S.D.	17.3	19.4	19.4	19.8
6-13	Mean	33.0	34.4	38.8	47.0**
	S.D.	19.2	19.3	21.0	19.0
13-19	Mean	48.7	55.6	62.4	70.4**
	S.D.	23.4	18.2	14.0	14.3
0-13	Mean	124.1	120.8	124.9	123.8
	S.D.	30.6	30.7	32.8	30.4

Mean Gain Over Weeks		Group and Sex 1F	2F	3F	4F
0-6	Mean	42.5	42.9	39.3	49.3*
	S.D.	14.1	11.9	13.7	16.4
6-13	Mean	18.7	20.0	25.2*	33.6***
	S.D.	8.5	8.7	13.1	10.1
13-19	Mean	18.4	18.0	17.8	30.0
	S.D.	8.4	12.0	7.7	20.9
0-13	Mean	60.5	64.8	65.5	87.1***
	S.D.	14.8	11.7	19.1	17.0

Values marked with asterisk differ significantly from controls
* $p < 0.05$
** $p < 0.01$
***$p < 0.001$

As indicated in the above tables, the high-dose group males gained weight relative to the controls for the periods of weeks 6 to 13 and 13 to 19. The high-dose group females gained more weight than the controls during all intervals (except that the weight gain for the period 13 to 19 was not statistically significant). During the period of weeks 6 to 13, the mid-dose group females gained more weight (39%) than the controls.

Increases (and decreases) in food consumption paralleled

the weight gain changes.

A NOEL is set at 10 mg/kg/day. At 60 mg/kg/day and above increases in body weight are noted in females. Body weight increases in males were statistically significant at 400 mg/kg/day only.

D. Ophthalmoscopy - Examinations were made at predose, for the interim kill animals at week 7, and the surviving animals at weeks 14 and 21. The eyes were examined with a Keeler direct ophthalmoscope following treatment with a mydriatic (1% tropicamide) agent.

No effects of lindane in the eyes of the treated rats were apparent following the ophthalmoscopic examinations.

[Note: For sections E and F below, blood samples were taken at necropsy following an overnight fast (no food or water).]

E. Hematology - The following parameters were investigated: hemoglobin, mean cell volume, red blood cell count and indices (mean cell hemoglobin, packed cell hemoglobin, packed cell volume, mean cell hemoglobin concentration), total and differential white blood cell count, and platelet count. In addition, a full myelogram was performed on a bone marrow smear.

The study report concluded and data were presented to show that "no consistent pattern of change to suggest a response to treatment in any of the parameters measured."

NOEL (for hematology) $\geq$ 400 mg/kg/day.

No summary table of the results of the bone marrow analysis was presented. Appendix 10 (pages 462-493) presents the individual animal data. Data were presented which showed that the rats for the interim, terminal (13-week) and recovery phase had bone marrow analysis. Nineteen parameters were quantitated in the bone marrow myelograms.

Comparison of the bone marrow myelograms did not indicate lindane treatment effects.

NOEL (for bone marrow analysis) $\geq$ 400 mg/kg/day.

F. Clinical Chemistry - The following parameters were investigated: glutamate oxaloacetate transaminase,

glutamate pyruvate transaminase, alkaline phosphatase, lactate dehydrogenase, gamma glutamyl transaminase, Na^+, Cl^-, inorganic phosphorus, blood urea nitrogen, creatinine, albumin, total cholesterol, K^+, Ca^{++}, glucose, total bilirubin, total protein, and albumin/globulin ratio.

Of these parameters, differences (statistically significant) were reported in the following: total cholesterol, plasma glutamate oxaloacetate, and glutamate pyruvate transferase.

1. Total Cholesterol - The study report (page 29) states that male groups 2, 3, and 4 had cholesterol concentrations that exceeded the controls on "day 1" but not on "day 2" and that cholesterol samples were taken on 2 days for the week 7 determinations. The only statistically significant increase in total cholesterol found in the summary table was during week 14. At this time, there was noted a 38 percent increase for the high-dose group females. Following the recovery phase, cholesterol levels in females were similar to those of the controls.

2. Plasma Glutamate Oxaloacetate Transferase (GOT/AST) and Glutamate Pyruvate Transferase (GPT/ALT) - These parameters were found to be elevated as much as 74 percent (for GPT/ALT) at week 7 for the high-dose group females. The mid-dose group values for these enzymes were 29 and 34 percent greater when compared with the controls. At week 14, the high-dose group had levels of these enzymes that were 40 and 33 percent higher than the controls. Although the elevations as indicated above were noted, statistical significance was not reported. It was also noted that the means for these data were accompanied by large standard deviations. In males, the high dose groups were comparable to the controls. At 20 weeks (following the recovery phase) the levels of these enzymes were reported as being within the range of the controls.

   Elevations in these enzyme levels are associated with various organ damage. Thus, the pathology of the liver, kidney, and other organs will be evaluated to determine if effects of lindane in these organs could have contributed to the increases in the serum levels of these enzymes.

NOEL (for clinical chemistry) $\geq$ 400 mg/kg/day elevations of some clinical parameters did not reach statistical significance).

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G. Urinalysis - Special water loading procedures were used in this study to help assess kidney function because the male rat kidney is a target organ for lindane toxicity. The water loading program consisted of administering by gavage 20 mL/kg of water just before initiation of urine collection. Urine samples were then collected overnight (18 hours) from animals deprived of food and water. In addition, when possible, urine was withdrawn mechanically from the bladder from all animals at necropsy. The following urinary parameters were investigated: volume, turbidity, specific gravity, protein*, ketones*, blood*, creatinine, color, pH*, glucose*, total bilirubin*, urobilinogen*, reducing substances*, urea, and microscopy of spun deposits. Creatinine clearance was also assessed. *Assessed semi-quantitatively by BM-Test-7 sticks.

The data (Appendix 7) were presented as individual animal results only and without a summary table. The study report states:

At week 7 -Treated males had higher incidence of positive scores for protein, blood and turbidity, and casts for the mid- and high-dose groups.

At week 14 -The low- and high-dose male groups had higher incidence of positive scores for blood than controls and all male treated groups had increased incidence of casts. The high-dose male group also had a higher incidence of positive scores for protein.

At week 20 -The incidence of casts in the high-dose male group was still in excess of the control group.

The following tables illustrate the results of the urinalysis for males.

Protein Scores*

Group	Interim	Terminal	Recovery
Control	8/12 = .67	7/20 = .35	0/10 = .00
Low	7/12 = .58	6/19 = .32	1/10 = .10
Mid	12/13 = .92	6/18 = .33	3/10 = .30
High	12/13 = .92	13/20 = .65	2/10 = .20

Blood Scores*

Control	8/12 = .66	18/20 = .9	8/10 = .80
Low	16/12 = 1.33	24/19 = 1.26	9/10 = .90
Mid	23/13 = 1.77	12/18 = .67	11/10 =1.10
High	21/13 = 1.62	26/20 = 1.30	7/10 = .70

Turbidity Scores*

Control	6/12 = .50	21/20 = 1.05	10/10 = 1.00
Low	12/12 = 1.00	23/19 = 1.21	10/10 = 1.00
Mid	17/13 = 1.31	19/18 = 1.06	10/10 = 1.00
High	12/13 = .92	23/20 = 1.15	11/10 = 1.10

Creatinine Clearance (mL/min)

	Interim	Terminal	Recovery
Control	.85 ± .21	.92 ± .26	1.03 ± .42
Low	.78 ± .20	.85 ± .22	.91 ± .29
Mid	.75 ± .18	.84 ± .25	.97 ± .15
High	.76 ± .13	.86 ± .25	1.00 ± .21

Casts (Animals Affected/Total Animals)and %

Control	1/12 (8.3)	3/20 (15)	2/10 (20)
Low	6/12 (50)	9/19 (47.4)	2/10 (20)
Mid	11/13 (84.6)	6/18 (33.3)	3/10 (30)
High	7/13 (53.8)	8/20 (40)	4/10 (40)

*Protein, bloqd and turbidity scores were determined by summing the number of + signs (numerator, an indication of the degree of severity) and dividing by the number of animals in the group.

In summary, TB-I concludes that the results of the urinalysis do not demonstrate any convincing evidence that lindane treatment affects the function of the kidney. The apparent increases in protein, blood,

turbidity, and casts do not have either the consistency over time or clearly defined dose responses to justify a cause and effect relationship with lindane dosing. For example, the histopathology data indicate that the lesions are more severe at 14 weeks, but the severity of the lesions does not parallel the apparent increases in these urinary parameters. In addition no effects were noted on creatinine clearance which is considered a more reliable estimate of the functional capacity of the kidney.

Inspection of the data for the females indicated slightly increased scores for blood (0.1 for the control and 0.18 for the high dose group at week 7 and 0.37 for the control and 0.56 for the high dose group at week 14), protein (0 for the control and 0.23 for the mid dose group and 0.27 for the high dose group at week 7). These scores for other times and dose groups as well as for turbidity were equivalent to the controls.

The study report, however, implies that <u>in males</u> the increases in protein, blood, and turbidity were treatment-related effects of lindane indicated by urinalysis.

NOEL (for urinalysis): UNRESOLVED.

The increases in blood, protein and turbidity scores in males were considered too indefinite to be conclusively related to lindane exposure. Resolution of the issue of an effect of lindane on these parameters would require <u>quantitative</u> analysis of the urine for blood, protein and turbidity rather than the semi-quantitative methods which use the relative indexes of measurement as +, ++ etc.

H. <u>Organ Weight</u> - The following organs were weighed following necropsy at each sacrifice time: adrenals, kidneys, ovaries, testes, brain, liver, spleen, and thymus. Paired organs were weighed separately.

Of these organs, the liver, kidneys, and adrenals were reported to have <u>increased</u> weights for various dosed groups. Of these three, the liver and kidney appeared to be affected by lindane, but the increase in adrenal weights was not convincingly related to lindane treatment.

1. <u>Liver Weight</u> - The following table illustrates the liver weight changes.

Absolute Liver Weight (g)

Group	Interim	Terminal	Recovery
Males			
Control	9.553	10.258	10.652
10 mg/kg/day	8.832	10.459	10.328
60 mg/kg/day	10.306 (7.9%) (R**)	10.417 (R**)	11.078
400 mg/kg/day	10.721* (12.2%) (R***)	11.831*** (15.3%) (R***)	11.965** (12.3%)
Females			
Control	6.649	6.894	6.817
10 mg/kg/day	6.690	6.880	7.107
60 mg/kg/day	7.161 (7.7%)	7.460** (8.2%) (R***)	7.130
400 mg/kg/day	7.984*** (20.0%) (R***)	9.658*** (31.4) (R***)	7.651 (12.2%)

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* p < 0.05
** p < 0.01
***p < 0.001
[Note: R in ( ) indicates statistical significance of the relative weights of liver to body weight.

The NOEL for increased liver weight is 10 mg/kg/day. Male liver weight remained statistically significantly elevated in the high-dose group (+12.3%) after the recovery period. Female liver weight in the high-dose group was also elevated after the recovery phase (+12.2%) but was not statistically significant.

2. Kidney Weight - The following table illustrates the kidney weight to body weight ratios. Relative kidney weights are illustrated rather than absolute weights because this more reliably reflects an indication of an effect.

Relative Kidney Weights

Group	Interim	Terminal	Recovery
Males			
Control	.3442	.3201	.3121
10 mg/kg/day	.3452	.3350 (+5%)^{NS}	.3214
60 mg/kg/day	.3612 (+5%)^{NS}	.3520 (+10%)***	.3005
400 mg/kg/day	.3672 (+7%)^{NS}	.3644 (+13.8)***	.3170
Females			
Control	.3578	.3525	.3234
10 mg/kg/day	.3505	.3534	.3296
60 mg/kg/day	.3572	.3579	.3369
400 mg/kg/day	.3482	.3405	.3229

***p < 001; NS = not significant
Note: Kidney weights are presented for the left and right kidneys individually in the study report. The above table presents the mean relative weights of both kidneys and the statistic derived from comparison of the individual left or right kidney with its respective control. Both left and right kidneys showed the same level of statistical significance (p < 0.001).

The NOEL for increased kidney weight is set at 10 mg/kg/day. The increase in kidney relative weight is evident in males only at the terminal sacrifice (after 13 weeks). The slight 5 to 7 percent increases in kidney weight for the males in the mid- and high-dose groups after 6 weeks did not reach statistical significance. The increase in kidney weight in males is demonstrated to be reversible since there was no increase after the recovery phase. Females were not affected.

3. Adrenal Weight - The study report states that there were increases in relative alone and/or relative and absolute adrenal weights for group 4 males and females, respectively, at the interim sacrifice of 8 to 14 percent. At the terminal kill the absolute weight of the male high-dose group adrenals was

elevated 9 percent and all female dose groups were elevated relative to the control group without an apparent dose response. Adrenal weights of all the dosed groups were within the control range of the recovery group rats. None of the increases reached statistical significance.

Overall, the NOEL for organ weight increase is set at 10 mg/kg/day. The LEL is 60 mg/kg/day, with both male and female groups having liver weight increases and males having kidney weight increases.

I. Gross Necropsy - Visual examination of the liver at the interim, terminal or recovery phases of the study did not indicate evidence of an effect of lindane treatment.

Visual examination of the kidney at the interim sacrifice revealed four incidences of "hydronephrosis" in the high-dose groups (31%) vs. only one incidence in each of the other three groups (8-9%). There was no apparent increase in this condition in either the terminal or recovery phases of this study.

Other internal organs were not considered to show an effect of lindane based on visual examination.

J. Histopathology - The histopathology report was presented in Appendix 13. It was signed by the Head of the Pathology Unit for the preparer of the document (D.S.G. Patton). The histopathology examination was confined to the rats in the control and high-dose test groups except for the femur and sternum (bone marrow analysis) and liver and kidney. For these tissues, all available animals were assessed. Complete histopathology was also performed on the decedents and gross necropsy observations were followed up microscopically. The following tissues are discussed individually.

1. Liver - Liver weight gain was increased in this study and the liver was demonstrated to be a target organ in other toxicity studies with lindane. There was also some evidence of liver alteration in this study as evidenced by the plasma level changes of cholesterol and serum enzymes.

The following table demonstrates the incidence of centrilobular hypertrophy in the liver of lindane-treated rats.

Centrilobular Hypertrophy*

	Interim		Terminal		Recovery	
	M	F	M	F	M	F
Control	0/9	0/7	0/20	0/16	0/10	0/9
10 mg/kg/day	0/9	0/9	0/19	0/14	0/10	0/10
60 mg/kg/day	8/10	4/10	10/18	8/17	0/10	0/10
400 mg/kg/day	10/10	8/8	20/20	13/13	0/9	0/7

*Rats with lesion/rats examined.

The above data show that the mid- and high-dose test groups at the interim and terminal (14-week) sacrifice have many more incidences of this condition. There were no rats reported to have centrilobular hypertrophy in the recovery groups. The testing laboratory recognizes that this condition is a result of lindane treatment.

The male livers of the rats in the recovery group also had 0/10, 1/10, 2/10, and 3/9 incidences of focal necrosis indicating a possible dose-dependent effect of lindane treatment. This possibility is noted by TB-1 although there was no evidence of dose-related increases in focal necrosis at the interim or terminal (14-week) sacrifice times. Nevertheless it is also noted that at the interim and terminal sacrifices neither the control nor low-dose test groups had this lesion type and there were singular incidences in the mid- and high-dose groups.

2. Kidney - The kidney relative weights were increased and urinalysis suggested a possible effect on the kidneys. The kidney is regarded as a target organ for lindane toxicity based on other study data with this insecticide.

The following tables show the status of male kidneys with regard to hyaline droplet formation (intensity), basophilic tubules (both regenerative and atrophic), tubular degeneration with necrosis, and for casts.

Male Kidneys*

Lesion	Group	Dose Level			
		Control	Low	Mid	High
Basophilic Tubules [Regenerative]	Interim	7/9	6/9	9/10	10/10
	Terminal	9/20	12/19	14/18	14/20
	Recovery	5/10	7/10	8/10	9/9
Basophilic Tubules [Atrophic]	Interim	3/9	3/9	7/10	8/10
	Terminal	8/20	13/19	14/18	17/20
	Recovery	5/10	6/10	9/10	8/9
Tubular Degeneration [Necrosis]	Interim	0/9	1/9	2/10	5/10
	Terminal	0/20	2/19	2/18	6/20
	Recovery	0/10	0/10	3/10	3/9
Casts	Interim	0/9	0/9	2/10	3/10
	Terminal	0/20	1/19	3/18	11/20
	Recovery	0/10	0/10	2/10	5/9

* Number of rats with lesion/number of rats examined.

Male Kidneys

Lesion	Group	Dose Level			
		Control	Low	Mid	High
Hyaline Droplet Formation (Severity Index)**	Interim	1.11 ± .78	1.67 ± 1.0	3.00 ± 0.0	3.35 ± .24
	Terminal	1.48 ± .81	1.86 ± 1.01	2.79 ± .69	3.40± .21
	Recovery	1.11 ± .93	0.80 ± 1.03	0.90 ± .88	1.00 ± .71

1985 data base and which contribute to the evaluation of Lindane as a carcinogen. These data were not used in CAG's current reevauation of the B2/C classification; however the CAG believes that these data deserve evaluation and could ultimately change the weight-of-evidence classification to a B2. We expect to review this data in CAG in the near future.

cc: CAG members
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