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

DEC 30 1992

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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

6(a)(2)

MEMORANDUM:

Subject: EPA ID # 009001: Lindane. Review of Reproduction Study in Rat (MRID # 422461-01)

EPA Record No. S417024
Caswell No. 527
P.C. No. 009001
HED Project No. D177595

From: Guruva B. Reddy, D.V.M., Ph. D. 12/29/92
Section 4
Toxicology Branch I
Health Effects Division (H7509C)

To: Larry Schnaubelt/Robert Richards
Project Manager 72
Reregistration Division (H7508W)

Thru: Marion P. Copley, D.V.M., D.A.B.T. Marion Copley 12/29/92
Section Head
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Health Effects Division (H7509C)

1. **CONCLUSIONS:**

The reproduction study in the rat was reviewed and determined to be core-Guideline.

This study satisfy the requirements of Subdivision F Guideline, 83-4 for the reproduction study in the rat.

A copy of the DER is attached.

2. **Action Requested:**

The Centre Internationale de Etudes du Lindane (CIEL) has submitted reproduction study in the rat in support of FIFRA 88. The study was reviewed and a copy of the DER is attached.

cc: Doherty

3. **Study reviewed:**

Study/Classification	TB-I Comments
83-4. Reproduction-2 generation Species: rat Life Sci. Res. Ltd,England, #91/CIL004/0948, Sept. 12, 1991 MRID #: 422461-01 core-Guideline	**Parental Systemic Toxicity NOEL** = 20 ppm (1.71 mg/kg/day) LEL is 150 ppm (13.05 mg/kg/day) based on decreased body weight gain in F_0 females during gestation. The NOEL (**alpha 2u globulin**) is 1 ppm (0.0865 mg/kg/day) and LEL is 20 ppm (1.71 mg/kg/day) based on increased absolute and relative kidney weights in F_0 animals and a slight increase in areas of change in the kidneys of F_1 males. Histologically, increased incidence of chronic interstitial nephritis, cortical tubular cell regeneration, hyaline droplets in proximal tubules, tubular necrosis with exfoliation and cellular casts and cortical tubular casts were observed. At 150 ppm, increased absolute and relative kidney weights and increased incidence of pale kidneys associated with area of change in F_0 and F_1 males; histologically the aforementioned incidences increased with dose. F_1 males also had increased incidence of hydronephrosis. Since alpha 2u globulin nephropathy is unique to mature male rats, it will not be used for regulating lindane. **Reproductive NOEL** = 20 ppm (1.71 mg/kg/day) LEL is 150 ppm based on decreased body weight gain and decreased viability up to PP4 in both generation offsprings, and delayed onset and completion of tooth eruption and completion of hair growth in F_2 generation pups. No reproductive parameters <u>per se</u> (fertility, mating, gestation) appeared to be affected by administration of lindane under the conditions of this study.

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Review by: Guruva B. Reddy, DVM, PHD 12/16/92
Review Section IV, Toxicology Branch I (7509C)
Secondary Reviewer: Marion P. Copley, DVM, DABT
Section Head, Review Section IV, Toxicology Branch I (H7509C) 12/24/92

DATA EVALUATION RECORD

Study Type: 2 generation repro.
Species: Rat
Guideline: 83-4

TOX. CHEM NO: 527

MRID NO.: 422461-01

P.C. NUMBER: 009001

TEST MATERIAL: Lindane

SYNONYMS: None

STUDY (REPORT) NUMBER: 91/CIL004/0948

SPONSOR: Centre Internationale de Etudes du Lindae (CIEL)
Brussels, Belgium

TESTING FACILITY: Life Science Res. Ltd
Suffolk, England

TITLE OF REPORT: Lindane: Reproductive Performance Study in Rats Treated Continuously Through Two Successive Generations

AUTHOR(S): V. C. King

REPORT ISSUED: September 12, 1991

CONCLUSION: **Parental Systemic Toxicity NOEL** = 20 ppm (1.71 mg/kg/day)
LEL is 150 ppm (13.05 mg/kg/day) based on decreased body weight gain in F_0 females during gestation.

The NOEL (**alpha 2u globulin**) is 1 ppm (0.0865 mg/kg/day) and LEL is 20 ppm (1.71 mg/kg/day) based on increased absolute and relative kidney weights in F_0 animals and a slight increase in areas of change in the kidneys of F_1 males. Histologically, increased incidence of chronic interstitial nephritis, cortical tubular cell regeneration, hyaline droplets in proximal tubules, tubular necrosis with exfoliation and cellular casts and cortical tubular casts were observed. At 150 ppm, increased absolute and relative kidney weights and increased incidence of pale kidneys associated with area of change in F_0 and F_1 males; histologically 3

the aforementioned incidences increased with dose. F_1 males also had increased incidence of hydronephrosis. Since alpha 2u globulin nephropathy is unique to mature male rats, it will not be used for regulating lindane.

Reproductive NOEL = 20 ppm (1.71 mg/kg/day)
LEL is 150 ppm based on decreased body weight gain and decreased viability up to PP4 in both generation offsprings, and delayed onset and completion of tooth eruption and completion of hair growth in F_2 generation pups.

No reproductive parameters per se (fertility, mating, gestation) appeared to be affected by administration of lindane under the conditions of this study.

Classification: core-Guideline

A. **MATERIALS:**

1. **Test compound:** Lindane, Description - white powder, Batch # - DA433, supplied by Rhone-Poulenc Agrochimie, Purity - not given (not available to the LSRL at the time of preparation of this report); however, an earlier study with same batch # listed purity of 99.5% (MRID # 414276-01), stability - stable at room temperature for 16 to 21 days.

2. **Test animals:** Species: rats, Strain: Charles River CD (Cesarian delivered), Age: 28 days, Weight (mean ± SE): male - 114 - 214 gm, females - 102 - 177 gm, Source: Charles River U. K. Limited, Margate, Kent, England; and acclimated for 2 weeks. The animals were maintained at a room temperature of 18 - 25°C, relative humidity of 40 - 70 % and light/dark cycles of 12:12 hours. The air was exchanged 15 times/hour.

B. **STUDY DESIGN:**

1. **Animal assignment**

Animals were assigned randomly to test groups (see Table 1). F_0 rats were fed the appropriate test diet throughout the entire study. An attempt was made to put a male and female from each F_1 litter into each treatment group. F_1 progeny in the experiment (F_2) were placed on the same diets as their parents immediately following weaning and continued until sacrifice. Dose levels were chosen based on results from an earlier study (LSR Report No. 90/CIL003/0515).

TABLE 1

Test Group	Conc. in diet %	(ppm)	F_0 (parents of f_1 pups) male	female	F_1 (parents of f_2 pups) male	female	Time Weighted Ave.* (mg/kg/day)
1 Cont	0.0	(0)	30	30	30	30	0
2 Low (LDT)	.0001	(1)	30	30	30	30	0.0865
3 Mid (MDT)	.002	(20)	30	30	30	30	1.71
4 High (HDT)	.015	(150)	30	30	30	30	13.05

* Calculated from weekly compound intake and averages during growth periods of study (N = 10)

Rats in the F_0 generation were maintained on the test diet for 71 days prior to mating (about 16 weeks of age). They were then were bred, to obtain the F_1 litters. These progeny were raised until weaning age (Postpartum (PP) day 21). Following a minimum of 70 day feeding period (13 weeks of age) the F_1 rats were bred to obtain the F_2 litters.

2. **Mating procedure**

Thirty males and females per group were used when possible, for each mating (see Table 1). Each female was mated to a non-sibling male from the same treatment group. When insufficient litters (F_1) were present to provide 30 males and 30 females in each group, additional offspring were randomly selected from litters selected randomly within each group. The pairs were checked daily for copulatory plugs and vaginal spermatozoa (by vaginal lavage). Females with confirmed matings were separated, and others were allowed a maximum of 3 weeks to achieve mating.

3. **Diet preparation**

Test diet was prepared weekly, and stored at room temperature in sealed polythene bags until used. It was tested for homogeneity and chemical stability. Samples of freshly prepared treated food were analyzed for chemical presence and concentration at the beginning and at 4-weekly intervals until termination of the study.

Results -

Homogeneity: Samples removed from 6 different locations in batches contained 95.4 to 97 % of target concentration. Means and standard deviations at each concentration were 0.97 ± 0.0719 ppm and 143 ± 10 ppm for 1 and 150 ppm, respectively.

Stability: Results from a preliminary study (LRS Report CIL/001/lindane) indicate that estimated shelf life of the test compound in diets was 16 and 21 days for 1 and 400 ppm, respectively; concentrations were 92 and 88.6 %, of target concentration, respectively, after 28 days storage at room temperature.

Concentration: Test compound concentrations in diet ranged from 88.7 to 91 % of target, at week 9 analysis. Means were 94.1 % (1 ppm), 91 % (20 ppm) and 88.7 % (150 ppm). The target concentrations in the diet at other time points were comparable to the week 9 results.

4. Animals received ground food (Biosure Laboratory Animal Diet No.2) and water ad libitum.

5. **Statistics -**

Data was analyzed for statistical significance relative to controls. Differences at the level of $P < 0.05$ or $P < 0.01$ were considered statistically significant.

Body weights, body weight change, litter size, food consumption and organ weights were analyzed by one way analysis of variance (ANOVA) and/or t-test.

Pre-coital interval and gestation length were analyzed by Mann-Whitney U-test.

Mating performance, conception rate, fertility index, gestation index, post-implantation survival index, live birth index, viability index, lactation index and sex ratios were compared by Chi-squared test, Fisher's Exact Probability test or Mann-Whitney U-test.

6. A signed quality assurance statement was attached.

C. **METHODS AND RESULTS:**

1. Observations:

Animals were inspected daily for signs of toxicity and mortality. A more detailed examination was conducted weekly.

Mortality - Mortality among F_0 and F_1 animals did not increase with dose and did not appear to be treatment-related. Among F_0 animals, one control female on Day 13 post coitum and one high dose male during Week 11 of pairing, were sacrificed for poor condition. Five F_1 animals died prior to sacrifice. Among two high dose females, one on Day 1 post partum (PP) due to prolapsed uterus and the second female on Day 7 PP for being in poor health were sacrificed. One control male was

cannibalized during Week 2 and a second male was sacrificed during Week 19 due to a tissue mass.

Toxicity - No treatment-related toxicity was reported.

2. **Body weight**

Males were weighed weekly until sacrifice. Females were weighed weekly during mating until conception; on gestation days (G) 0, 6, 13, and 20 and on PP 1, 4, 7, 14, 21 and 25.

F_0 males - Mean body weight and weight gain of treated animals were similar to controls during the study.

F_0 Females - Mean body weight gain was significantly reduced (11 %) by Day 20 of gestation, in high dose females (Table 2). Also at this dose, the mean body weight gain of females on Day 1 of lactation was significantly lower, when compared to the controls, but recovered by weaning (not shown in table). The mean body weight gains were not statistically significant among treated and control animals during the growth phase.

Table 2. **Mean Body Weight Gain (g) of F_0 Females**[a] (No. Animals)

Study Period (weeks)	**Dose** (ppm)			
	0	1	20	150
Growth (1 - 10)	177 (30)	178 (30)	184 (30)	172 (30)
Gestation (10 - 13)	162 (29)	158 (30)	161 (29)	144 (28)**
Lactation (13 - 17)	5 (29)	2 (30)	5 (28)	16 (25)

[a] Data extracted from Study Tables 3, 11 and 13; Body weight gain information presented here was calculated from the aforementioned tables.
** Significantly different from controls, $P < 0.01$

F_1 males: The initial (6 weeks old) and terminal (19 weeks old) body weights of high dose males were significantly ($P < 0.05$) lower than those of controls (Appendix 1); these differences were sporadic, lacked dose-relationship and there was no treatment-related change in body weight gain. Therefore, the above statistical significances in body weights are considered to be of equivocal significance. Body weights/body weight gains of the 1, 20 and 150 ppm groups throughout the F_1 generation were similar to the controls.

F_1 females: The mean body weights/body weight gains of all

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treated females were not affected by treatment during the growth (before pairing) phase of the study.

Gestation and Lactation: The mean body weights of treated females were unaffected by treatment, except the group 2 (1 ppm) females displayed increased body weights throughout the gestation and lactation periods.

3. **Food consumption food efficiency and compound intake**

Food consumption was determined weekly for males and females only until pairing. Food efficiency and compound intake were calculated using the mean weekly food consumption, group mean body weight and theoretical dietary concentration.

Food consumption -

During growth - There were no differences in food consumption between F_0 control and treated animals in males or females, except for a slight reduction (6.3 %; $P \leq 0.01$) in high dose females during the first week of treatment. Food consumption of F_1 males in 20 and 150 ppm groups were marginally reduced by 6.5 % and 7.4 %, respectively, during the week 3 and 8 % during the week 4 (150 ppm) of treatment. These decreases are marginal and not consistent, therefore, considered to be of no biological significance. In F_1 females, the food consumption was not affected by treatment.

Food efficiency - During growth - Food conversion efficiency of both sexes in F_0 and F_1 generations were unaffected by treatment.

Compound intake (time weighted averages) during the 70 day growth periods, for the males and females combined for both generations is listed in Table 1. Compound consumption was proportional to theoretical dose level increments in each treatment group. **Compound consumption for F_0 and F_1 generations during the gestation and lactation periods were not determined.**

4. **Reproductive effects**

Gestation length and number of live and dead progeny were determined on the day of delivery. Females that did not deliver were examined for evidence of pregnancy.

Mating performance and fertility -

The following indices were based on each breeding trial and are shown in the table below-

Mating index - proportion of pairs showing evidence of

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mating/total number of pairs.

Fertility index - Proportion of pairs with evidence of mating that resulted in pregnancy/total number of pair.

Mating indices and fertility indices are presented in Table 3. No significant differences among control and treatment groups were observed. Mating indices ranged from 93% to 100% and fertility indices 79% to 100%.

Table 3. **Mating and Fertility Indices**[1]

Generation : Mating	**Dose** (ppm)			
	0	1	20	150
F_0: F_1 Mating/ Fertility	30/30 (100%) 30/30 (100%)	30/30 (100%) 30/30 (100%)	29/30 (97%) 29/30 (97%)	29/29 (100%) 27/29 (93%)
F_1: F_2 Mating/ Fertility	27/29 (93%) 23/29 (79%)	29/29 (100%) 28/29 (97%)	30/30 (100%) 28/30 (93%)	30/30 (100%) 27/30 (90%)

[1] Data extracted from study Tables 9 and 37

The following were based on the combination of 2 trials/ 2 generations are shown in the table 4.

Female fertility index - Proportion of females with evidence of mating that had at least one mating resulting in pregnancy/total females with evidence of mating.

Male fertility index - Proportion of males with evidence of mating that had at least one mating resulting in pregnancy/total males with evidence of mating.

Table 4. **Male and Female Fertility Indices**[1]

Generation	**Sex**	**Dose** (ppm)			
		0	1	20	150
F_0: F_1 mating	♂ ♀	30/30 (100%) 30/30 (100%)	30/30 (100%) 30/30 (100%)	29/29 (100%) 29/29 (100%)	27/29 (93%) 28/30 (93%)
F_1: F_2 mating	♂ ♀	23/27 (85%) 23/28 (82%)	28/29 (97%) 28/29 (97%)	28/30 (93%) 28/30 (93%)	27/30 (90%) 27/30 (90%)

[1] Data extracted from study Tables 9 and 37

Fertility indices for each sex did not appear to be affected by treatment at any dose level. The majority (79% to 100%) of animals mated at the first oestrus.

Gestation parameters-

The gestation length was comparable in all treatment groups and for each generation in the study. Mean gestation length varied between 22 - 23.5 days. Two F_0 females one each in Group 3 and Group 4 gave births after 24 days and 25 days, respectively. The delayed births were considered spurious.

The gestation survival or post-implantation index - proportion of total no. of offsprings born/total no. of implantation sites.

The gestational survival index was not affected by treatment at any dose tested for both generations. The indices ranged from 86% - 93%. There was slight reduction in post-implantation index in the Group 4 F_2 generation animals. The indices for the Groups 1, 2, 3 and 4 were 93, 89, 93 and 86%, respectively. The decreases were not dose-related, therefore, considered to be of no biological significance.

Liveborn index - proportion of pregnant females that delivered live progeny/total pregnant females.

Live born indices are presented below in Table 5. There was no difference in the indices for both generations.

Table 5. **Liveborn Indices**[1]

Generation	Dose (ppm)			
	0	1	20	150
F_0: F_1 Mating	29/30 (97%)	30/30 (100%)	29/29 (100%)	28/28 (100%)
F_1: F_2 Mating	23/23 (100%)	28/28 (100%)	28/28 (100%)	26/27 (96%)

[1] Data extracted from Tables 12 and 40

5. **Lactation effects (Progeny measurements)**

Surviving neonates were counted on days PP1, 4, 7, 14, 21 and 25. On day PP4 the pups were sexed and litters were randomly culled to 8 pups, 4 males and 4 females when possible.

Litters and individual pups were weighed on days PP1, 4, 7, 14, 21 and 25, respectively. The survivors were sexed again on day PP14 and 25.

Neonatal survival: No treatment-related differences in lactation survival rates, post-implantation survival index, live birth index or in mean litter size were observed. Data is presented in Appendix 1 taken from the study tables (Group mean litter sizes and Post-implantation survival, live birth, viability and lactation indices). Offspring viability and litter size at PP4 were slightly reduced for high dose F_1 progeny due to death of three litters. In 150 ppm F_2 progeny, the post-implantation survival index and viability index upto Day 4 post partum were slightly reduced; the latter was due to two litters died or killed for humane reasons.

Neonatal body weights are provided in Appendix 6 taken from study tables. Body weights decreased significantly in all high dose (150 ppm) pups (F_1 and F_2 generation) at Day 1 and Day 25 post partum, when compared to the controls. At other dose levels body weights were lower than controls but were not significant when compared to the controls. The data was not analyzed by sex.

Sex distribution was not affected appreciably by administration of test compound. Ratios of males:females varied between 0.85 to 1.05 and variations were not dose-related. The number of males and females at birth are shown in Appendix 2.

Offspring development, as assessed by the timing of onset and completion of pinna unfolding, hair growth, tooth eruption and eye opening were not affected by treatment in F_1 generation pups. In F_2 generation, the onset and completion of tooth eruption and completion of hair growth were delayed 10.5, 11.6 and 24%, respectively, when compared to the concurrent controls, at 150 ppm, and the differences were statistically significant ($P \leq 0.01$). The aforementioned growth parameters were also delayed in comparison to the background controls. These effects were considered treatment-related and can be used to regulate the chemical.

6. **Sacrifice and Pathology**

Progeny that died prior to scheduled sacrifice (<21 days old) and Progeny that were culled on day 4 were given a gross external and internal examination. Specimens of abnormal tissues were saved.

F_0 - These rats were sacrificed approximately three weeks after weaning of the F_1 progeny, at about 36 weeks of age. All were given complete gross necropsies and the liver, epididymis, kidneys, ovaries, prostrate, seminal vesicles, testes and uterus with cervix were weighed. Livers, kidneys and reproductive tissues, including vagina, uterus, ovaries, mammary glands, testes, epididymis, seminal vesicles and prostate, were fixed for histologic examination. Of those offspring that were not used for breeding were given a gross external and internal examination.

F_1 - Those that were used for parenting (F_1) the F_2 litters were sacrificed (about 36 weeks of age) approximately three weeks after the F_2 progeny were weaned. The necropsy and tissue sampling procedures followed were similar as in the case of F_0 animals. Females that failed to mate, mated but did not give birth or whose litters died before weaning were killed with the majority of females and were given a complete gross necropsy as described above. Of those offspring that were not used for breeding were given a gross external and internal examination.

F_2 - All were sacrificed at weaning and were given a gross external and internal examination for abnormalities.

a. Organ weight (Parental) Organ weight changes are presented below in Table 6.

F_0 parental animals: In males, the absolute and relative kidney weights increased significantly ($P \leq 0.01$), when compared to the controls, at 20 and 150 ppm . The increases were dose-related. In addition, the absolute liver weights of males given 150 ppm also increased, when compared to the controls. The increases were marginal and was probably a compensatory mechanism due to administration of xenobiotics, therefore, considered to be of no biological significance. In females, the absolute and relative liver weight increased significantly in the 150 ppm group, when compared to the controls and are considered to be no biological significance for the reasons cited above. In addition, in females, at 150 ppm, the relative kidney weight increased, however, the increase was marginal, therefore, considered to be of no biological significance..

F_1 parental animals: The relative liver and absolute and relative kidney weight of males receiving 150 ppm increased 8.3, 11.9, and 18.5%, respectively, when compared to the controls; the increases were statistically significant. In females, in 150 ppm group, a treatment-related increase in the relative liver and kidney weights were observed. The relative liver weights in males and relative liver and kidney weights in females are considered to be of no biological

significance as explained above.

Table 6. **Organ Weights**[1]

Organ Wt. (g)	Males (ppm)				Females (ppm)			
	0	1	20	150	0	1	20	150
F_0 Parental								
Abs. Liver	23.6	22.4	22.3	25.1	13.3	13.3	13.5	14.5[a]
Rel. Liver (%)	3.48	3.28	3.40	3.68[a]	3.60	3.57	3.66	4.03[b]
Abs. Kidney	4.45	4.58	4.81[b]	5.53[b]	2.69	2.64	2.80	2.83
Rel. Kidney (%)	0.67	0.67	0.74[b]	0.82[b]	0.73	0.71	0.76	0.79[b]
F_1 Parental								
Abs. Liver	25.2	26.5	24.1	25.5	13.8	14.5	13.4	14.8
Rel. Liver (%)	3.39	3.47	3.34	3.67[a]	3.52	3.64	3.57	4.02[b]
Abs. Kideny	4.77	4.85	4.89	5.34[b]	2.74	2.80	2.68	2.83
Rel. Kidney (%)	0.65	0.65	0.68	0.77[b]	0.70	0.71	0.72	0.77[b]

[1] Data extracted from Study tables 23 to 26 and 51 to 54
[a] = P≤ 0.05, [b] = P ≤ 0.01

b. Gross pathology

Parental - Pathological changes were only seen in male kidneys (Table 7). Adult males in the 150 ppm group exhibited increased incidence of pale kidneys and with areas of change. In addition, an increased incidence of hydronephrosis was observed in high dose males. In males, at 20 ppm, there was a slight increase in the incidence of areas of change in kidneys. Females kidneys did not exhibit any gross morphological changes observed in males.

Table 7. **Summary of Organ Macropathology**[1]								
Kidney	**Male** (ppm)				**Female** (ppm)			
F_0 Parental								
Pale	0/30	0/30	1/30	10/29[b]	0/29	0/30	0/30	0/30
Areas of change	2/30	0/30	2/30	7/29	0	0	0	0
Hydro-nephrosis	2/30	0/30	1/30	1/29	0	0	0	0
F_1 Parental								
Pale	0/28	0/30	2/30	10/30[b]	1/30	0/29	0/30	0/28
Areas of change	1/28	1/30	4/30	5/30	0	0	1	0
Hydro-nephrosis	0/28	2/30	2/30	7/30[a]	0	0	0	0

[1] Data extracted from Study tables 22 and 50
[a] = $P \leq 0.05$, [b] = $P \leq 0.01$

Progeny - The incidence of hydronephrosis and hydroureter in the F_1 generations offsprings is presented below in Table 8.

Table 8. **Summary Incidence of Hydronephrosis and Hydroureter in F_1 offsprings**[1]												
Group	**Dying before Termination** (ppm)				**Culled on Day 4 PP** (ppm)				**Unselected at Termination** (ppm)			
	0	1	20	150	0	1	20	150	0	1	20	150
A	6 (6)	12 (9)	28 (8)	55 (13)	190 (28)	173 (29)	186 (77)	126 (21	169(29)	178(30)	158 (28)	132 (24)
B	3:3	8:4	14: 14	27: 28	84: 106	96: 77	108 :77	64: 62	82: 87	90: 88	80: 78	67: 65
C	0	0	3.6	21. 9	0	2.3	3.8	4.0	4.7	8.4	3.8	4.6
D	0	0	3.6	14. 5	1.6	2.3	5.9	4.0	3.0	2.8	1.3	3.1

Key : A = # Examined (# of litters), B = # Males:Females, C = Hydronephrosis (unilateral + bilateral), D = Hydroureter (unilateral + bilateral)
[1] EXtracted from Summary Tables 19, 20 and 21

The overall incidence (%) of hydronephrosis/hydroureter in 1, 20 and 150 ppm groups were 5.23/2.5, 3.7/3.7, and 7.3/5.4, respectively, compared to the 2.2/2.2 of controls.

Histopathology was not done on these tissues. The data were not analyzed statistically. The figures expressed as hydronephrosis and hydroureters are combination of unilateral and bilateral incidences. The increased incidence of hydronephrosis/hydroureters in the 150 ppm group was mainly due to higher incidence in the offsprings which died before termination. The increase incidence of hydronephrosis and hydroureters is marginal and lacked the dose-relationship, therefore, considered to be of no biological significance.

c. Microscopic pathology (Parental only) -

Treatment-related kidney and liver changes are shown in Appendix 3. F_0 and F_1 males in mid and high dose (20 and 150 ppm) groups exhibited increased incidence of chronic interstitial nephritis, cortical tubular cell regeneration, hyaline droplets in proximal tubules, tubular necrosis with exfoliation and cellular casts and cortical tubular casts, characteristic of accumulation of alpha2u-globulin associated with treatment of hydrocarbons. The aforementioned histological changes were statistically significant, except for cortical tubular casts in F_1 males, which were dose-related. These changes were not observed in female rats. An interim report titled "Immunohistochemical localization of $alpha_2u$-globulin (a2ug) in kidneys of rats treated with lindane" by James Swenberg and Daniel Dietrich observed a dose-related increase in the accumulation of $alpha_2u$-globulin in the proximal tubules of nephron. These accumulations at the junctions of the proximal tubules and the thin limbs of Henle were hyaline droplets of varying sizes ... multifocal cortical tubular necrosis of varying intensity, cortical tubular regeneration and granular casts. Based on the aforementioned study, the CIEL has concluded that since $alpha_2u$-globulin is unique to male rats and has not been shown to be produced by humans, the regulation of lindane based upon kidney pathological changes in male rats appears inappropriate. Currently, the Agency has adopted the mechanistic approach and the kidney lesions seen above are considered unique to mature males rats and will not be used to regulate the chemical (EPA Risk Assessment Forum Document - $Alpha_{2u}$ -Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat, EPA/625/3-91/019F, September 1991).

In the liver, an increased incidence of periacinar hepatocellular hypertrophy was seen in both F_0 and F_1 high dose males. In addition, in the F_1 males, the periacinar hepatocellular hypertrophy increased, at 20 ppm, when compared to the controls. All the aforementioned changes were statistically significant. The authors considered that hepatocellular hypertrophy was an adaptive change which has been commonly associated with administration of xenobiotics

and therefore, was not of any biological significance. The authors assumptions are appropriate.

D. **DISCUSSION**

The NOEL for parental systemic toxicity is 20 ppm (1.71 mg/kg/day)
LEL is 150 ppm (13.05 mg/kg/day) based on decreased body weight gain in F_0 females during gestation.

The NOEL (**alpha 2u globulin**) is 1 ppm (0.0865 mg/kg/day) and LEL is 20 ppm (1.71 mg/kg/day) based on increased absolute and relative kidney weights in F_0 animals and a slight increase in areas of change in the kidneys of F_1 males. Histologically, increased incidence of chronic interstitial nephritis, cortical tubular cell regeneration, hyaline droplets in proximal tubules, tubular necrosis with exfoliation and cellular casts and cortical tubular casts were observed. At 150 ppm, increased absolute and relative kidney weights and increased incidence of pale kidneys associated with area of change in F_0 and F_1 males; histologically the aforementioned incidences increased with dose. F_1 males also had increased incidence of hydronephrosis. Since alpha 2u globulin nephropathy is unique to mature male rats, it will not be used for regulating lindane.

Reproductive NOEL = 20 ppm (1.71 mg/kg/day)
LEL is 150 ppm based on decreased body weight gain and decreased viability up to PP4 in both generation offsprings, and delayed onset and completion of tooth eruption and completion of hair growth in F_2 generation pups.

No reproductive parameters per se (fertility, mating, gestation) appeared to be affected by administration of lindane under the conditions of this study.

This study appears to be well conducted and is satisfactory for regulatory purpose. One deficiency noted was that the chemical purity of the compound was not provided; however, an earlier study with same batch # listed purity of 99.5 % (MRID # 414276-01). The study is therefore classified as core-Guideline.

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