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

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DEC 30 1992

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id# 009001. Lindane: Review of the rat chronic feeding/carcinogenicity study submitted January 1991.

TOX CHEM No.: 527
PC No.: 009001
TOX PROJECT No.: 1-1395/D164902
Submission No.: S397012

FROM: John Doherty, Ph.D., D.A.B.T. for Copley 12/24/92
Section IV, Toxicology Branch I
Health Effects Division (H7509C)

TO: Robert Richards/Larry Schnaubelt
Product Manager Team #72
Special Review and Registration Division (H7508C)

THROUGH: Marion Copley, DVM, Section Head Marion Copley 12/24/92
Section IV, Toxicology Branch I
Health Effects Division (H7509C)

I. CONCLUSION

The rat chronic feeding/carcinogenicity study (submitted January 4, 1991 under MRID No.: 418537-01) was reviewed and assigned SUPPLEMENTARY classification. Additional histopathology must be provided on the adrenal gland and historical control data related to the spontaneous occurrence of pheochromocytomas in this gland must be provided so that TB-I can complete its assessment (see comment 2).

Lindane was shown to affect the male kidney but this phenomena was demonstrated to be manifested through the induction of alpha 2u globulins. This specific type of lesion in the male rat kidney is not currently regarded as being relevant to human health risk assessment (see item 3, 4 under comments).

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II. Action Requested/Background

The McKenna and Cuneo Law Offices on behalf of their client the Centre International d'Etudes du Lindane (CIEL) has submitted in response to a prior data-call-in a rat chronic feeding/carcinogenicity study (series 83-5, Life Science Research study No.: 90/CIL002/0839, Nov. 7, 1989, MRID No.: 418587-01). The study was reviewed by Toxicology Branch I (TB-I) and the following comments apply.

III. Toxicology Branch Comments

1. The study was classified as SUPPLEMENTARY. A copy of the DER is attached.

2. TB-I was not able to complete its assessment of the significance of the increase in pheochromocytomas in the high dose group. In order to upgrade the study, TB-I requests that the registrant provide additional histopathology on the adrenal gland from the animals in the groups dosed with 1, 10 and 100 ppm of lindane.

In addition, TB-I requests that historical control data for the frequency of pheochromocytoma in (male and female, separately) Wistar strain rat used for this study be submitted. Historical control data should be, as far as possible, from the same laboratory and should be presented on a study by study basis (not as a total number of tumors per total number of animals examined). The study dates, number of animals assessed and information (as far as possible) on the age of the animals with the tumors should be presented. The data for benign and malignant pheochromocytomas, separately and combined, is required.

3. The LEL (100 ppm) is based on liver pathological changes. TB-I recognizes that 10 ppm is a possible threshold level but more consistent effects are noted at the next highest dose level of 100 ppm. The magnitude of the effects at 10 ppm does not justify assignment of this level as the LEL.

4. The effects of lindane on the male rat kidney were attributed to lindane's association with alpha 2_u globulins (refer to HED Document No.: 007859 dated April 10, 1990). Current Agency policy provides that this type of effect is not related to human health risk assessment (refer to document EPA/625/3-91/019F, September 1991, Risk Assessment Forum monograph entitled "Alpha 2_u globulin : Association with Chemically Induced Renal Toxicity and Neoplasia in the Male rat). Thus, the liver toxicity induced by lindane is regarded by TB-I as the critical toxicity endpoint defined by this study.

2.

4. There was no evidence of induction of liver tumors although lindane is currently regarded as a B_2-C carcinogen based on liver tumors in mice based on an earlier classification system.

C. **Study Reviewed**

Study Identification/ Classification	
83-5. Chronic Feeding/Carcinogenicity-rats. Life Science Research, Study No.: 90/CIL002/0839, Nov. 7, 1989. MRID No.: 418587-01 Classification: SUPPLEMENTARY. Additional histopathology of the adrenal gland and historical control data must be provided in order to complete assessment.	**Tentative** Systemic Effects: (non-alpha 2u globulin) NOEL/LEL = 10/100 ppm: 100 ppm: Periacinar hepatocyte hypertrophy; liver and spleen weight increase and decrease in platelets. 400 ppm: decreased survival in females (trend in males); convulsions in females; decrease in body weight gain (early) and increase in inorganic phosphorous, calcium, urea and cholesterol and decrease in albumin/globulin ratio and decrease in RBC parameters. Carcinogenicity: Additional data on adrenal gland necessary to complete assessment. Kidney Effects: LEL < 1 ppm for kidney effects associated with induction of alpha 2u globulins. Endpoint not to be used for regulatory toxicology. Dose Levels Tested: 0, 1, 10, 100 and 400 ppm in diet corresponding to 0, 0.05, 0.47, 4.81 nd 19.66 mg/kg/day for males and 0.06, 0.59, 6.00 and 24.34 mg/kg/day for females. Wistar strain rat.

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Reviewed by: John Doherty, Ph.D. 12/22/92
Section IV, Tox. Branch (H7509C)
Secondary reviewer: Marion Copley, DVM 12/24/92
Section IV, Tox. Branch (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 83-5. Chronic feeding/carcinogenicity-rats

MRID NO.: 418537-01

TOX CHEM No.: 527
PC No.: 009001

TEST MATERIAL: Lindane (gamma-hexachlorocyclohexane), 99.6-99.75% purity, lot No.: DA433 (batch CIL/002).

STUDY NUMBER(S): 90/CIL002/0839.

SPONSOR: Centre Internationale des Etudes du Lindane (C.I.E.L.)

TESTING FACILITY: Life Science Research, England

TITLE OF REPORT: "Combined Oncogenicity and Toxicity Study by Dietary Administration to Wistar Rats for 104 Weeks".

AUTHOR(S): S.J. Amyes, Ph.D.

REPORT ISSUED: November 7, 1989.

In Life Phrase: Treatment commenced on October 28, 1987 and terminal sacrifice was on October 31, 1989.

CONCLUSIONS:

Systemic Effects: (non-alpha 2_u globulin) NOEL/LEL = 10/100 ppm: 100 ppm: Periacinar hepatocyte hypertrophy; liver and spleen weight increase and decrease in platelets. 400 ppm: decreased survival in females (trend in males); convulsions in females; decrease in body weight gain (early) and increase in inorganic phosphorous, calcium, urea and cholesterol and decrease in albumin/globulin ratio and decrease in RBC parameters.

Carcinogenicity: Additional data on adrenal gland necessary to complete assessment.

Kidney Effects: LEL < 1 ppm for kidney effects associated with induction of alpha 2u globulins. Endpoint not to be used for regulatory toxicology.

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Dose Levels Tested: 0, 1, 10, 100 and 400 ppm in diet corresponding to 0, 0.05, 0.47, 4.81 nd 19.66 mg/kg/day for **males** and 0.06, 0.59, 6.00 and 24.34 mg/kg/day for **females**. Wistar strain rat.

Classification: SUPPLEMENTARY. Additional sections and readings of the adrenal gland from the rats in the low and mid dose groups are required for completion of the review. In addition, data on the historical control frequency for pheochromocytomas in the Wistar strain rat are considered necessary to complete the assessment of the possibility of an association between lindane dosing and tumors in the adrenal. The study is a candidate for upgrading.

Quality Assurance Statement: Provided. Signed by D.L.M. Weller. Good Laboratory Practice Statement: Provided.

REVIEW

[Note: An interim report for the 30 day and 26 week sacrifices was reviewed (refer to HED document No.: 7461, dated Aug.30, 1989). This report indicated that there were more deaths in the female high dose group, periacinar hypertrophy was present in the liver (males at 100 ppm, both sexes at 400 ppm) and liver weight was increased in the 400 ppm dose group. The male kidney was affected at dose levels as low as 10 ppm with the condition of this organ in males being more severe as the dose level increased. This present review is concerned primarily with the oncogenicity phase and 52 and 104 week toxicity phases but also includes the 78 week sacrifice of the animals in the recovery phase.]

Experimental Constants:

Test Material:

Chemical:	Lindane
Source:	Rhone-Poulenc, Agrochimie
Batch:	DA 433
Purity:	99.75% gamma isomer of hexachlorocyclohexane

Test System:

Species:	Rat
Strain:	Wistar
Supplier:	Charles River Breeding, Kingston, New York.
Age:	21-28 days of age on arrival,
Weight:	Males: 88-156 gms. Females: 82-146 gms
Feed:	Labsure LAD-2 rodent diet, ad libitum
Water:	ad libitum

B. STUDY DESIGN:

Table 1 outlines the experimental design for this study.

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Table 1. Experimental design (including dose levels and compound intake) of the chronic feeding/oncogenicity study with lindane.

	Sacrifice Interval					mg/kg/day[5]	
Dose Group	30 days	26 weeks	52 weeks	78 weeks[3]	104 weeks[4]	Males	Females
Control	15[1]	15	15	15	55[2]	--	--
1 ppm	15	15	15	15	55	0.05	0.06
10 ppm	15	15	15	15	55	0.47	0.59
100 ppm	15	15	15	15	55	4.81	6.00
400 ppm	15	15	15	15	55	19.66	24.34

1. Number of rats/sex per group.
2. Five rats per sex were designated for analysis of their tissue for lindane content.
3. This group was dosed with lindane for 52 weeks and allowed 26 weeks to recover from any reactions prior to sacrifice.
4. Oncogenicity aspect of the study (50 rats/sex/dose group).
5. These data were taken from Table 7B of the study report (pages 110 to 111) and are compound intake means for the oncogenicity phase of the study. In the study report the values are given as the group mean but no where in Table 7B is the term "mg/kg/day". The study report on page 35 under methods states that the achieved dosage was mg/kg bodyweight/day.

Analytical Chemistry:

Stability: Data on the stability of 1 and 400 ppm lindane in the test feed were presented. For the 1 ppm test diet, the initial concentration of 1.06 ± 0.22 ppm, dropped to 0.96 ± 0.034 after 7 days, 0.85 ± .006 after 14 days and was 0.92 ± 0 after 28 days. According to the report, the estimated shelf life is 16 days. The 400 ppm test diet was initially 386 ± 7, for means of two determinations was 355, 344 and 355 at days 7, 14 and 28 respectively. The estimated shelf life is 22 days.

Homogeneity: Samples from six different positions were taken from the 1 and 400 ppm test diets. These revealed a mean concentration of 0.99 ± 0.099 with a 10% coefficient of variation
for the 1 ppm dose group and 388 ± 7.9 with a 2% coefficient of variation for the 400 ppm dose group.

Concentration: The results of 12 analyses taken over the two year course of the study of the sample diets for concentration were preserted. These indicated an achieved concentration of 107 ± 19%, 96 ± 10%, 93 ± 8% and 95 ± 4% of the 1, 10, 100 and 400 ppm test diets.

Conclusion. The stability, homogeneity and concentration analytical data indicate that these parameters were in an acceptable range.

Statistics: Table 2 illustrates the statistical tests used for this study.

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Table 2. Statistical tests used in study.

Test	Parameter assessed
Student's "t" test using pooled within group error variance.	Body weight and gain, hematology (except eosinophiles, basophils, monocytes, normoblasts which are not normally distributed). blood chemistry, and urinalysis.
Bartlett's test for homogeneity Dunnett's test, when Bartlett's test not significant. Behrens-Fisher test, when Bartlett's test is significant.	Organ weight
Cox's test (time to event as applied as an overall test for homogeneity of survival curves and for pair wise comparison against controls) Tarone's extension of Cox's test to examine linear trend on dose and to assess deviation for linearity. Two analyses performed, one with and one without animals killed for humane reasons.	Time to event (mortality).
Fisher's exact probability As a two tailed test. As a one tailed test.	 Distribution of macroscopic or microscopic (non-neoplastic) pathological entities. For neoplastic microscopic entities for apparent increases in incidence with treatment.
Life Tables Incidental tumor analysis Unadjusted analysis (Fisher's exact and Cochran-Armitage test) (above as approach advocated by the National Toxicology Program)	For tumor incidence for phaeochromocytoma, pancreas exocrine cell tumors, testicular interstitial cell tumor and pituitary adenoma

METHODS AND RESULTS:

1. **Observations.** Animals were said to be inspected twice daily for signs of toxicity and mortality and handled once weekly for palpation.

Mortality (survival). [Note: the 26 week interim report indicated that there were more deaths in the high dose female (8) group than in the controls (2), refer to review of the interim sacrifice.]

The survival pattern for the males and females in the oncogenicity phase of this study and illustrated in Table 3

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below.

Table 3. Survival pattern in the oncogenicity phase of the lindane study. [Data abstracted from Table 2B of the study report.]

	Males		Females	
Dose Group	Week of 50% Survival[2]	Deaths/ Survivors[1]	Week of 50% Survival	Deaths/ Survivors
Control	92	35/20	104	28/27
1 ppm	98	35/20	99	34/21
10 ppm	94	38/17	100	31/24
100 ppm	89	44/11ss	96	36/19
400 ppm	93	46/9ss	89	45/10ss

ss: statistically significant, ns = not significant (study report conclusion).
1. Cumulative number of animals dying by week 105/number of survivors after 105 weeks based on 55 animals (including the analytical experiment) in each group per sex.
2. The first week having 27 deaths.

There is no indication of a compound related effect in survival through weeks 93 for males but there is an apparent trend for decreased survival after that time for the 100 and 400 ppm dose groups. For females, the 100 (possibly) and 400 ppm dose groups have an apparent decrease in survival time. The high dose female group was statistically significantly decreased with regard to survivors. This is consistent with the interim report.

Conclusion (survival): NOEL/LEL = 100/400 ppm. 400 ppm: deaths in the female group. Apparent trend in the male group.

Clinical signs. Convulsive episodes were noted in females in the high dose group with there being 3/3, 1/1, 1/1, 2/2 and 11/27 animals affected/incidents of episodes. This is consistent with the higher rate of deaths in this sex although there were no indications that the convulsions preceded the deaths.

Conclusion (clinical signs): NOEL/LEL = 100/400 ppm.. 400 ppm: convulsions in females.

2. Body weight. [Early and transient body weight gain decreases (NOEL/LEL = 100/400 ppm) were discussed in the review of the interim report.]

Animals were weighed weekly for the first 16 weeks and then at four week intervals thereafter. Table 4 below depicts body weight and body weight gain at selected intervals.

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Table 4. Body weight and body weight gain at selected intervals. [Data abstracted from Table 3 of the study report].

Interval		Dose Level[1] Control	1 ppm	10 ppm	100 ppm	400 ppm
0-1	M	61/7	60/6	61/8	58/6	46/10***
	F	28/5	28/6	27/7	23/6***	11/6***
0-12	M	337	343	345	328	311
	F	148	149	153	146	142
13-52	M	231	250	243	254	223
	F	146	133	16.	141	111
56-104	M	-92	-61	-54	-102	-90
	F	52	49	66	29	75
Final 0-104	M	762/134, 571/130[1]	747/131 560/129	750/103 560/96	689/91(-10%) 499/95	651/80(-14%) 468/76*
	F	513/120 361/118	506/80 353/82	558/107 404/104	491/94 336/92	508/38 353/42

1. Data are body weight gain (gms)/standard deviation (when presented).
2. Data are mean body weight (gms)/standard deviation.
* p < 0.05, * p < 0.01 and *** p < 0.001 Students "t" test, study report statistics.

Table 4 confirms that most of the weight gain effects were early in the study but the final weight of the males was -10% (not significant) and -14% ($p < 0.05$) for the 100 and 400 ppm dose groups. Final body weight for the females were essentially the same for all dose groups.

The study report maintains that during the recovery phase the high dose group gained more than the controls. Inspection of the data indicate that there were large variations in the weight gain data (standard deviations for mean weight gains were 50% of the means) and only females in the high dose group (120 $\pm$ 58 gms gained) probably gained more weight than the controls (92 $\pm$ 44 gms gained). The recovery weight gain data are, however, not considered critical to interpretation of the entire study.

Conclusion (Body weight and gain): NOEL/LEL = 100/400 ppm. 400 ppm: Male weight decreased in high dose group at termination. Initial body weight gain decrease in first three weeks of study.

[Note: Initial body weight decrease at 100 ppm in females and terminal body decrease in males are not considered of sufficient magnitude to justify assigning a LEL at 100 ppm.]

3a. Food consumption. Food consumption was measured at the

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same intervals as the body weight. A decrease (15% males and 19% females) was evident for week one and a smaller decrease was noted for at least the first 12 weeks of the study. Total food consumption for weeks 0 to 104 was 99 to 100% for the males and 101 to 102% for the females when compared to the controls.

3b. Compound intake. Compound intake was calculated based on food consumption and compared to the body weights of the rats. The achieved dosage for the oncogenicity phase of the study is presented in Table 1.

4. Ophthalmological examinations: Assessments were made prior to commencement of treatment with lindane and during weeks 4, 25, 52, 78 (for the recovery phase) and 104 and all (including the toxicity and oncogenicity phases but not the rats in the proof of absorption phase) rats were reportedly examined.

No compound related findings were reported.

5. Hematology and Clinical Chemistry.

Blood was collected before treatment (25/sex) and at weeks 3, 12 and 24 of treatment from the group assigned for sacrifice after 25 weeks (10/sex). Another sampling was made at week 51 from the group to be sacrificed at week 52. A sampling was made at week 77 for the recovery groups scheduled for sacrifice at week 78. Finally a sampling (20/sex) was made at week 103 for the terminal sacrifice group. The blood was sampled from the retro-orbital sinus following overnight fasting and under light ether anesthesia.

The CHECKED (X) parameters were examined.

a. Hematology

	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*		Mean corpuscular HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpuscular volume (MCV)
x	Platelet count*		Reticulocyte count
	Blood Clotting Measurements	x	Packed cell volume
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

Hematology The study report asserts a LEL of 100 ppm for increases in platelets and a LEL for decreases in hemoglobin

concentration, erythrocyte counts and packed cell volume. These four parameters are discussed separately as follows.

a. Platelets. Increases in platelet counts were significantly increased at weeks 12 and 24 as indicated in Table 5 below.

Table 5. Platelet counts (1000/cm). [Data are abstracted from Table 9 of the study report].

		Dose Level				
Interval	Sex	Control	1 ppm	10 ppm	100 ppm	400
12 weeks	M	561(33)	515(34)	544(82)	569(40)**	568(69)**
	F	555(43)	586(37)	539(72)	531(54)	599(44)
24 weeks	M	417(47)	428(38)	451(50)	477(48)**	499(38)***
	F	406(57)	445(37)	384(52)	461(52)*	462(61)*

*p < 0.05, ** p < 0.01 and *** p < 0.001 (study report statistics Students t test.

Other intervals did not show statistical differences. The increases at 12 weeks are small (1%) but are slightly larger at 24 weeks (14.4% at 100 ppm and 19.7% at 400 ppm for males). Females are increased at week 24 only being 13.5% and 13.8% for the 100 and 400 ppm dose groups respectively. TB-I accepts the registrants conclusion for an effect at 100 ppm but notes that only a small deviation is noted and this does not persist throughout the experiment as indicated by there not being other statistical differences.

b. Hemoglobin concentration. The maximum decrease in hemoglobin concentration was noted for both males (-15.6%, p < 0.01) and females (-17.6%, p < 0.001) at week 104. At this interval the mid dose group was essentially equivalent to the controls. Other instances of decreased hemoglobin concentration were evident at week 3 (females 6.6%, p < 0..001) and at other intervals less than 6% (p < 0.05 or 0.01). The occasionally significant deviations at 100 ppm or lower were not supported by similar increases or decreases at the higher dose level.

c. Erythrocyte counts. The pattern was essentially similar to the hemoglobin concentration above with the maximum decrease at week 104 with both males (-14.1%, p < 0.05) and females (-21%, p < 0.001) showing decreases.

d. Packed Cell volume. Again the high dose group was affected most at week 104 with the males (-15.9%, p < 0.01) and females (-18.2%, p < 0.001) showing decreases.

CONCLUSION (Hematology): NOEL/LEL = 10/100 ppm. At 100 ppm: increases in platelets. At 400 ppm: Red blood cell parameters

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decreased.

b. Clinical Chemistry. Sampled at the same time the hematology assessments were made.

	Electrolytes:		Other:
x	Calcium	x	Albumin
x	Chloride	x	Blood creatinine
	Magnesium	x	Blood urea nitrogen
x	Phosphorous	x	Cholesterol
x	Potassium		Globulins
x	Sodium	x	Glucose
	Enzymes	x	Total Bilirubin
x	Alkaline phosphatase	x	Total Serum Protein
	Cholinesterase		Triglycerides
x	Creatinine phosphokinase		Serum protein electrophoresis
x	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)		
x	Serum aspartate aminotransferase (also SGOT)		
x	gamma glutamyl transferase		
	glutamate dehydrogenase		

Note: items not checked not considered critical.

The study report asserted that compound related increases in the first year of treatment were evident for inorganic phosphorous (Pi), Ca++ in both sexes and total plasma cholesterol and urea and lower albumin/globulin ratio. The percent increase (or decrease, relative to the controls for the high dose group only is illustrated in Table 6.

Table 6. Statistically significant increases in the 400 ppm dose group as compared to the controls for selected clinical chemistry parameters. [Data are abstracted from Table 10 of the study report.]

	Pi		Ca++		Cholesterol	Urea	Alb/glo
Interval	Males	Females	Males	Females	Females	Females	Females
3 weeks	7.3%*	18.2%***	–	10%***[1]	45%***	54%***	-8.3%*
12 weeks	8.1%*	29***	3.4%**	3.5%**	53%***	31%***	-18.2%**
24 weeks	13.8%***[3]	ns	3.8%**	3.6%*	67%	20%**	-10%**
51 weeks	17.6%***	38.5***	ns	7.8%***[2]	110%***	41.1%	-18.2%
Recovery	ns	ns	ns	ns	ns	ns	ns
104 weeks	ns	ns	ns	ns	ns	ns	ns
Pretest	5/0.3 mEq/l	4.8/0.3	5.5/0.2 Meq/l	5.7/0.2	101/12 mg%	35/8 mg%	1.4/0.1 ratio

1. All dose groups were $p < 0.001$ greater (6-10%) than the control probably indicating that the control (5.0±0.2) was low for this sampling.
2. The 1, 10 and 100 ppm dose groups are also elevated 3.8% ($p < 0.05$ or 0.01)
3. The 100 ppm dose group is also elevated 6.9% ($p < 0.05$)

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CONCLUSION (clinical chemistry): NOEL/LEL = 100/400 ppm. At 400 ppm increases in inorganic phosphorous, calcium, cholesterol and urea and decease in albumin/globulin ratio.

6. Urinalysis (refer to Appendix 1.

7. Sacrifice and Pathology. All control and high dose animals were sacrificed on schedule and all animals at all doses that died were reportedly subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

	X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic
x	Tongue	x	.Aorta	xx	.Brain
x	Salivary glands	xx	.Heart	x	Periph. nerve
x	Esophagus	x	.Bone marrow	x	Spinal cord
x	Stomach	x	.Lymph nodes	xx	.Pituitary
x	Duodenum	xx	.Spleen	x	Eyes (inc optic nerve
x	Jejunum	xx	.Thymus		Glandular
x	Ileum		Urogenital	xx	.Adrenals
x	Cecum	xx	.Kidneys*		Lacrimal gland
x	Colon	x	.Urinary bladder	x	Mammary gland
x	Rectum	xx	.Testes	x	.Parathyroids
xx	Liver	x	Epididymides	xx	.Thyroids
	Gall bladder	x	Prostate		Other
xx	Pancreas	x	Sominal vesicle	x	Bone
Respiratory		xx	Ovaries	x	Skeletal muscle
x	Trachea	x	.Uterus	x	Skin
xx	Lung				
x	bronchi				
	Nose				

x	All gross lesions and masses*
x	Presumptive tumors

The oviduct and salivary glands were not among the organs/tissues listed in the pathology reports.

8. Organ weight. The following organs have weight variations as described.

A. Liver. The study report maintains that the high dose males and females had absolute and relative liver weight increases on all occasions other than on completion of the reversibility period although statistical significance was not achieved in every case.

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Table 7. Liver weight (absolute and relative) increases over the two year period in the chronic feeding/carcinogenicity study with lindane. [Data abstracted from Tables 17 to 21.]

			Dose Level[1]			
Weight	Sex	Interval	1 ppm	10 ppm	100 ppm	400 ppm
Absolute	M	30 days	--	--	--	--
		26 weeks	--	--	--	+40.8%**
		52 weeks	--	--	--	+15.9%ns
		Recovery	--	--	--	--
		104 weeks	+2.5%ns	+6.6%ns	+11.2%ns	+21.2%**
Absolute	F	30 days	--	--	--	+29.2%**
		26 weeks	--	--	--	+32.3%**
		52 weeks	--	--	--	+21.2%ns
		Recovery	--	--	--	-7.6%ns
		104 weeks	+8.0%ns	+3.7%ns	+8.6%ns	+31.6%**
Relative	M	30 days	--	--	--	+14.0%*
		26 weeks	--	--	--	+34.7%**
		52 weeks	--	--	--	+20.3%**
		Recovery	--	--	--	--
		104 weeks	--	--	+14.4%**	+38.5%**
Relative	F	30 days	--	--	--	+37.2%**
		26 weeks	--	--	--	+31.7%**
		52 weeks	--	--	--	+44.2%**
		Recovery	--	--	--	-6.2%ns
		104 weeks	--	--	17.6%*	+33.5%**

* $p < 0.05$, ** $p < 0.01$ and ns = not significant. Dunnett's test or Behren's-Fisher test. -- essentially the same as the control.
1. Data are expressed as percent relative to the control.

B. Spleen weight. Table 8 below illustrates the variation in spleen weight noted throughout this study.

Table 8. Spleen weight data. [Data are extracted from Tables 17 to 21].

				Dose Level[1]		
nterval	Weight	Control	1 ppm	10 ppm	100 ppm	400 ppm
30 days	Abs	0.619/.79				0.709/.147)
	Rel	0.265/.03)	0.2915/.04	0.2777/.04	0.3069/.33	0.3222/.,05
26 weeks	Abs	0.616/.07				0.719/.10
	Rel	0.1717/.024	0.1894/.018	0.1954/.029	0.1892/0.26	0.2071/.023
52 weeks	Abs	0.759/.21	0.844/,125	0.816/0.51	0.914/.200	0.999/.154*
	Rel	0.178/.043	0.208/.03	0.1967/.02	0.199/.06	0.2753/.03**
ecovery	Abs	1.141/.21				1.099/.29
	Rel	.2225/.036				0.2213/.041
04 weeks	Abs	1.281/.527			1.243/.28	1.683/.641
	Rel	0.2531/.08			0.2632/.05*	0.3393/.128

. Data are weight in grams/standard deviation for absolute weight and the relative eights/standard deviation. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ study report tatistics. Bartlett's test for homogeneity and Dunnet's test.

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CONCLUSION (organ weights): NOEL/LEL = 10/100 ppm. At 100 ppm liver and spleen weight are increased.

c. Microscopic pathology. The protocol called for microscopic examination of all animals in the scheduled sacrifices and those dying during the study for the control and high dose groups only. Only certain tissues/organs (kidney. liver, lung, testis, spleen, bone marrow and gross lesions) were assessed for the 1, 10 and 100 ppm dose groups.

A. Liver. Liver weight was increased. Both male and female rats had compound related increases in periacinar hepatocytic hypertrophy. Table 9 illustrates the occurrence of this lesion throughout the experiment.

Table 9. Frequency and occurrence periacinar hepatocytic hypertrophy.

		Incidence				
Interval	Sex	Control	1 ppm	10 ppm	100 ppm	400 ppm
30 days	N	10/8	10/10	10/10	10/10	10/9
	M	0	0	0	7**	10***
	F	0	0	0	2	9**
26 weeks	N	9/10	10/10	10/10	10/10	10/9
	M	0	0	2	8**	10***
	F	0	0	0	2	9***
52 weeks	N	10/10	10/10	10/9	10/9	9/8
	M	0	3	3	9***	9***
	F	0	0	0	5*	8***
Recovery	N	8/8	8/9	9/8	7/9	8/9
	M	0	0	2	0	1
	F	0	0	1	5*	2
104 weeks	N	50/50	50/50	50/50	50/50	50/50
	M	1	0	6	25***	40***
	F	1	1	4	19***	43***

++ p , 0.01, *** p < 0.001. N = Number of males/females examined.

There was only a single incident of a rat with a liver tumor (as hepatocellular adenoma or carcinoma), this was a high dose female. There was also one incident each of anaplastic carcinoma (a control male), hemangiosarcoma (low dose group female) and cholangioma (high dose group male).

CONCLUSION (liver pathology): NOEL/LEL = 10/100 ppm. The 10 ppm

dose level is recognized by TB-I as being a possible threshold dose level for periacinar hepatocyte hypertrophy but the magnitude and consistency of the response does not justify assigning 10 ppm as a definite effect level or LEL.

B. Adrenals. Among the males there was an apparent increase in phaeochromocytoma as indicated in Table 10 below.

Table 10. Adrenal phaeochromocytoma.

		Incidents		
Dose level	N	Benign	Malignant	Total
Control	50	7	0	7 (14%)
1 ppm	34	4	0	4 (12%)
10 ppm	36	5	2	7 (19%)
100 ppm	42	3	3	6 (14%)
400 ppm	50	12	1	13 (26%)

For these data, when benign tumors alone are considered, the Life Table Test for "fatal analysis" is positive ($p < 0.05$) but other statistical tests for fatal or incidental analysis were not significant. When the benign and malignant tumors are combined, the Life Table Test ($p < 0.01$) and Cochran-Armitage Test for trend ($p < 0.05$) for fatal analysis and the Prevalence Test ($p = 0.047$) for incidental analysis are all positive. None of the test dose levels were positive for pair wise comparison when assessed by the Fisher Exact Test for either fatal or incidental analysis.

CONCLUSION (adrenal pathology): TB-I cannot at this time make its final conclusions regarding a possible effect in the adrenal. The indication of a possible response at the high dose level calls for preparation and microscopic assessment of all of the animals in the 1, 10 and 100 ppm dose groups. In addition, historical control data on the background incidence of pheochromocytoma in the Wistar rat should be provided to assist in determining a possible effect of lindane in the adrenal. See details in Discussion section of DER.

C. Spleen and bone marrow. Lindane has been implicated as a causative agent for aplastic anemia. In this study certain blood elements were decreased and spleen weight was increased.

There were no definite pathological finding in the spleen to support the blood element or organ weight changes. Among the females, the incidence of "extramedullary haemopoiesis" was 11, 13, 17, 14 and 18 for 50 animals in each dose group for the control to high dose group respectively. All male groups had 4-7 incidents with the high dose having only 4. TB-I does not consider this condition to be definitely compound related in the females.

Bone marrow smears were made from the animals at each sacrifice interval. The parameters reportedly investigated were mean myeloid to erythroid ratio and cellularity and composition and for incidence of fatty marrow. Two bone marrow samples were prepared from each animal. They were fixed in methanol and stained with Wright's stain and Pappenheimer's stain. No evidence of compound related effects was evident.

9. DISCUSSION: The following Table provides a comparison between the conclusion in the study report with those made by TB-I.

Study Report	TB-I Assessment
Clinical Signs: NOEL/LEL (clinical signs) = 100/400 ppm. 400 ppm: convulsions in females.	Concurs.
Mortality: NOEL/LEL = 100/400 ppm. 400 ppm: decreased survival among females. Trend present for decreased survival for males at 100 and 400 ppm.	Concurs.
Body Weight: females sig, males higher	NOEL/LEL = 100/400 ppm. 400 ppm early decrease (first 3 weeks) in both sexes. Terminal body weight for males decreased.
Ophthalmoscopy: No effects.	Concurs.
Hematology: NOEL/LEL = 10/100 ppm for platelet effects; NOEL/LEL = 100/400 for erythrocyte effects.	Concurs.
Clinical Chemistry: NOEL/LEL = 100/400 ppm.	Concurs. At 400 ppm increases in inorganic phosphorous, calcium, cholesterol and urea and decease in albumin/globulin ratio.
Urinalysis: See under special studies.	
Organ Weight: NOEL/LEL = 10/100 ppm.	NOEL/LEL = 10/100 ppm. At 100 ppm liver and spleen weight are increased.
Non-neoplastic Pathology: (non alpha 2_u globulin) NOEL/LEL = 10/100 ppm	NOEL/LEL = 1/10 ppm. 10 ppm is recognized as a threshold level for increased periacinar hepatocyte hypertrophy.
Neoplastic Pathology: No evidence of carcinogenicity.	Additional readings on 1, 10 and 100 ppm dose groups for the adrenal are required to complete assessment. Historical control information on pheochromocytoma also requested.

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Special Assessments: Special investigations made for assessment of kidney function.	NOEL/LEL (kidney function) = 100/400 ppm. At 400 ppm increased urine volume and change in specific gravity.
Overall Conclusion for NOEL/LEL: NOEL = 10 ppm.	NOEL/LEL = 10/100 ppm. Liver histopathology is primary effect.
Adequacy of dosing.	Dose levels considered adequate for carcinogenicity evaluation.

CONCLUSION (study): This study is classified as SUPPLEMENTARY. The study is a candidate for upgrading pending receipt and review of the additional data on the adrenal gland.

1) Conduct histologic examination of all adrenals in the low and two mid dose groups.

2) TB-I requests that historical control data for the frequency of pheochromocytoma in the Wistar strain rat (males and females) used for this study be submitted. Historical control data should be, as far as possible, from the same laboratory and should be presented on a study by study basis (not as a total number of tumors per total number of animals examined). The study dates, number of animals assessed and information (as far as possible) on the age of the animals with the tumors should be presented. The data for benign and malignant pheochromocytomas, separately and combined, is required.

Systemic Effects: (non-alpha 2_u globulin) NOEL/LEL = 10/100 ppm: 100 ppm: Periacinar hepatocyte hypertrophy; liver and spleen weight increase and decrease in platelets. 400 ppm: decreased survival in females (trend in males); convulsions in females; decrease in body weight gain (early) and increase in inorganic phosphorous, calcium, urea and cholesterol and decrease in albumin/globulin ratio and decrease in RBC parameters.

Carcinogenicity: Additional data on adrenal gland necessary to complete assessment.

Kidney Effects: LEL < 1 ppm for kidney effects associated with induction of alpha 2u globulins. Endpoint not to be used for regulatory toxicology.

Dose Levels Tested: 0, 1, 10, 100 and 400 ppm in diet corresponding to 0, 0.05, 0.47, 4.81 nd 19.66 mg/kg/day for males and 0.06, 0.59, 6.00 and 24.34 mg/kg/day for females. Wistar strain rat.

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Appendix I. Special Assessment of the kidney.

The male rat kidney is particularly susceptible to certain halogenated hydrocarbons and other compounds. This response includes the build up of alpha 2_u globulins and subsequent lesions in the kidney. The interim report of this study (HED Document No.: 7461) and a subsequent request from TB-I to actually analyze the kidney tissue for alpha 2_u globulins already indicated that lindane should be classified in this special model (refer to HED Document No.: 007859, dated April 10, 1990 from Marion Copley, DVM to Margaret Rice).

The original design of this study included special assessment of kidney function which were made at the request of the Agency. The methods and results of both the routine and special kidney assessments are kept separately from the main part of this DER for two reasons. First, to give an idea of how lindane specifically effects the kidney and how the effects were demonstrated. The second reason is because he alpha 2_u globulin like kidney lesions are not considered relevant to human health risk assessment (refer to document EPA/625/3-91/019F, September 1991, Risk Assessment Forum monograph entitled "Alpha 2_u globulin : Association with Chemically Induced Renal Toxicity and Neoplasia in the Male rat).

The interim sacrifice report (refer to HED Document No.: 007461) indicated that after 30 days and 26 weeks there was some indication that functional capacity of the kidney was affected based on increased urine volume, water consumption, decreased specific gravity and indications of increased urinary protein. The emphasis of the following discussion is on effects after the week 26 assessment.

Overview of Assessments for Kidney Effects On/In Rat Kidney.

1. Urinalysis.

Urine was collected from fasted animals at prior to treatment, at weeks 3, 12, 24, 51, 77 (recovery) and 103. The CHECKED (X) parameters were examined.

X		X	
x	Appearance*	x	Glucose*
x	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*	x	Nitrate(nitrite)
x	Protein*	x	Urobilinogen (urobilin)
x	Urea output		
x	Creatinine output		
x	Total reducing substance		

Urine volume for the male group dosed with 400 ppm

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remained higher at week 51 (33%) and at week 104 (22%). Urine volume was equivalent to the control group after the recovery phase. Other urinary parameters at 1 and 2 years were considered spurious.

2. Water Loading

At approximately 8 AM, the rats were given 20 ml/kg of water by gavage and placed in metabolism cages for collection of their urine for the next eight hours.

No effects were noted after 1 and 2 years.

3. Water deprivation

At about noon time, each rat was placed in an individual metabolism cage under conditions of food and water deprivation. After about four hours urine was removed from each rat by supra-pubic pressure. This residual urine was discarded and the rat returned to its metabolism cage without food or water for an overnight period and the urine was collected.

The males in the high dose group were reported to produce a greater volume of urine with a lower pH than the controls on all occasions.

4. Terminal collection

When possible prior to sacrifice (either in extremis or at scheduled sacrifice), urine was collected by supra-pubic pressure and after sacrifice by direct removal from the bladder (this was pooled with the sample taken prior to necropsy).

No test chemical effects were noted.

5. Creatinine and urea clearances

This was assessed at pretest and after 3, 12, 24, 51, 77 (recovery) and 103 weeks of treatment. Plasma and urinary levels (from routine sample collections) of endogenous creatinine and urea were determined.

The report stated that "creatinine and urea clearance of males was not impaired by treatment.

6. Pathological findings in the kidneys

The following conditions in the males noted in the interim report were also noted in the 52 week and 104 week sacrifices.

Hyaline droplets in the proximal tubules. Table 1a illustrates the presence and recovery of several conditions noted in the male

kidneys after 26 weeks of exposure (i.e at weeks 52, 77 and 104).

Table 1a. Hyaline droplets in proximal tubules in the later phases of the study.

Lesion	Week[1]	Control	1 ppm	10 ppm	100 ppm	400 ppm
Hyaline Droplets in the proximal tubules	52	0/10	0/10	10/10***	10/10***	9/9***
	77	4/8	5/8	7/9	7/7	2/8
	104	10/18	6/18	10/16	9/9*	7/9
	104	17/50	11/50	23/50	23/50	23/50

1. Week 77 is the recovery phase. Week 104 (second entry) includes all animals in the oncogenicity phase.

Tubular regeneration. This condition was not mentioned in the tables for male kidney effects for weeks 52, 77 or 104.

Interstitial chronic nephritis. This condition was only occasionally present at weeks 52, 77 and 104 and not in a dose related manner.

Cortical tubular necrosis. Only one incident was indicated after week 26. This was a high dose male.

Papillary Mineralization. This condition was not indicated as being compound related in the interim report. However, 5 of 10 and 7 of 9 of the 100 and 400 ppm dose groups were affected at week 52 (both groups being statistically significant and no incidents in the other groups) . This condition was still present after the recovery in the 10 (1 of 9), 100 (2 of 7) and 400 (4 of 8) but statistical significance was not attained. After 104 weeks the 100 (3 of 9) and 400 (4 of 9) dose groups had this condition (statistically significant) while other groups did not.

Kidney Tumors. There were only three tumors in the kidneys, all in the high dose groups. There were a total of two lipomas, one each in the male and female high dose group. The third tumor was a transitional cell carcinoma in a high dose group male.

CONCLUSION (kidney effects): The overall NOEL/LEL is 1/10 ppm and is based on the kidney effects noted primarily in the interim sacrifice. Refer to HED Document No.: 007461 for discussion. There is no evidence that the kidney condition progresses beyond the condition described for the first 26 weeks. There was no evidence that the alpha 2u globulins increase resulted in an increase in kidney tumors.

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Appendix 2. **Lindane Residues in tissues.**

The blood, brain, liver and kidney samples from five rats from each group were assessed for lindane content at weeks 4, 27, 53, 80 (recovery) and 105. Very little lindane was detected in the animals assessed at week 80 which were not dosed with lindane for 27 weeks. Thus, lindane does not remain in the tissue for that extended period. Each of the tissues assessed is discussed separately below.

Table A1 below compares the levels of lindane as determined at week 53 for the blood, brain, liver and kidney samples.

Dose Level	Lindane concentration [1] Serum		Brain		Liver		Kidney	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	6±2	4±2	20±18	38±3	ND	ND	.07±.02	.03±.02
1 ppm	59±9	30±14	45±4	53±3	364±109	298±53	1.5±.58	0.18±.07
10 ppm	201±47	112±34	105±17	306±84	1052±251	1470±296	7.9±1.4	0.83±.16
100 ppm	1203±566	926±393	957±161	2339±566	2054± 2096	5869± 5208	75±24	4.6±1.3
400 ppm	1418±471	3192± 2144	1420±313	5932± 1971	1709±743	12404± 8403	148±54	9.1±2.6

1. Data are ng/ml for blood, ug/gm for kidney and ng/gm for liver and brain.

Blood. The blood data indicated high standard deviations (50% or higher) and these high standard deviations obscured making generalizations. However, it appears that throughout the study the blood levels were near those of the one year analysis as presented above. No clear sex difference was established.

Brain. Females had distinct higher levels at least for the 100 and 400 ppm dose groups throughout the study. Distinct progressive increases in brain levels were not noted throughout the 2 year experiment.

Liver. Similar to the brain, females had higher levels for the 100 and 400 ppm dose groups. High standard deviations precluded further generalizations but an increase with time for the females was apparent.

Kidney. the male kidney had a consistent and marked higher concentration than for females for the 10, 100 and 400 ppm dose groups. The highest concentration in the male kidney was noted at week 4 (272.2 ug/gm) with this value progressively declining to 28.2 ug/gm by week 105. The maximum concentration in the female kidney was 38.2 ug/gm at week four and again there was a decrease to a value of 7.24 ug/gm at week 105.

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Lindane
Life Science Research
96/CLL 002/0839
Nov. 7, 1989
MRID No.: 418587-00

009909

83-5 Chronic Feeding/Oncogenicity in the Rat

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 50 rats/sex/group (3 test groups and control group).
3. ✓ Dosing duration is at least 24 months.
4. ✓ Number of survivors in any group does not fall below 50% at 18 months or 25% at 24 months.
5. N/A Doses tested include an MTD or limit dose if nontoxic (1000 mg/kg).
6. • ✓ Doses tested include a NOEL.
7. • ✓ Analysis for test material stability, homogeneity and concentration in dosing medium
8. ✓ Individual daily observations.
9. ✓ Individual body weights.
10. ✓ Individual or cage food consumption.
11. • ✓ Opthalmoscopic examination (at least pretest and at term) control and high dose.
12. ✓ Clinical pathology data for at least 10 rats/group consisting of 13, 14 & 15
13. ___ Hematology at 6 month intervals consisting of at least; ✓ Mean corpuscular Volume
    - ✓ Erythrocyte count
    - ✓ Hemoglobin
    - ✓ Hematocrit
    - ✓ Leucocyte count
    - • ✓ Differential count
    - ✓ Platelet count (or clotting measure)
14. ___ Clinical chemistry at 6 month intervals consisting of at least;
    - ✓ Alkaline phosphatase
    - ✓ Aspartate aminotransferase
    - • ND Creatinine kinase
    - ✓ Lactic dehydrogenase
    - ✓ Glucose
    - ✓ Bilirubin
    - ___ Cholesterol
    - • ✓ Creatinine
    - ✓ Total Protein
    - ✓ Albumin
    - ✓ Urea
    - ✓ Inorganic phosphate
    - ✓ Calcium
    - • ✓ Potassium
    - ✓ Sodium
    - • ✓ Chloride
15. ___ Urinalysis at 6 month intervals consisting of at least;
    - ✓ Blood
    - ✓ Protein
    - ✓ Ketone bodies
    - ✓ Appearance
    - ✓ Glucose
    - ✓ Total bilirubin
    - • ✓ Urobilirubin
    - ✓ Sediment
    - ✓ Specific gravity (osmolality)
    - • ✓ Volume

    ✓ pH
    ✓ Total urea
    ✓ Total creatinine
16. ✓ Individual necropsy of all animals. Extensive examinations
17. ✓ Histopathology of the following tissues performed on all nonrodents and rodents, all control and high dose animals, all animals that died or were killed on study, all gross lesions on all animals, target organs on all animals and lungs, liver and kidneys on all other animals.
    - ✓ aorta
    - ✓ jejunum
    - ___ peripheral nerve (Sciatic)

Criteria marked with a • are supplemental and may not be required for every study.

✓ eyes	✓ bone marrow	✓ kidneys†
✓ caecum	✓ liver†	✓ esophagus
✓ colon	✓ lung†	✓ ovaries†
✓ duodenum	✓ lymph nodes	oviduct
✓ brain†	✓ stomach	✓ pancreas
✓ skin	✓ mammary gland	✓ rectum
✓ heart†	✓ spleen†	✓ spinal cord (3x)
✓ testes†	✓ musculature	✓ thyroid / parathyroids
✓ pituitary	✓ epididymis	salivary glands
✓ ileum	✓ adrenals†	✓ thymus
✓ trachea	✓ uterus	✓ urinary bladder

† organs to be weighed.

‡ The position document entitled "Selection of a Maximum Tolerated Dose (MTD) in Oncogenicity Studies (EPA No. 540/09-88-003) stated EPA's criteria for determining if an oncogenicity study has been adequately performed in terms of doses tested. However OPP is also aware that older oncogenicity studies, upon initial review or re-review, may have been tested at doses lower than the predicted MTD. In the event that such testing appears to be at doses less than the predicted MTD, the Office of Pesticides Program has been reviewing and considering the entire weight of the evidence to determine if retesting is necessary. Certain factors which affect the agency's decision to retest include but are not limited to the following: demonstrated oncogenicity in another species, nearness to the apparent MTD, genotoxic effects, structure-activity factors, absolute value of the highest dose tested and metabolic considerations.

✓ brain
✓ prostate
✓ seminal vesicle

Criteria marked with a * are supplemental and may not be required for every study.