UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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SEP 27 1991

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id. # (No number provided): "Technical". Lindane: Review of a 13 week dermal toxicity study with rabbits including a 6 week interim sacrifice and 6 week recovery phase. Study includes special assessment of the kidney and bone marrow myelograms.

TOX CHEM No.: 527
TOX PROJECT No.: 0-1040
Record No.: 262401

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CONCLUSION

The rabbit dermal toxicity study with lindane did not indicate the presence of the specific kidney lesions (although male kidney weight was elevated slightly) which are found in the male rat following lindane treatment. Thus, this study supports the hypothesis that lindane induces a specific lesion in the male rat kidney but other species are not affected. Although some decreases (<9%) in the high dose group were apparent on hemoglobin, RBC counts and packed cell volume at week 13, no effects of lindane on the bone marrow blood elements were noted. Thus, lindane was not considered to be associated with a specific anemia. The NOEL/LEL for this study was set at 10/60 mg/kg/day based on liver alterations and adrenal weight increases.

This study was determined to be CORE GUIDELINE for an 82-3 subchronic (90 day) dermal toxicity study. No additional subchronic dermal toxicity testing is required at this time.

Background

In response to a previous request from Toxicology Branch, Health Effects Division (refer to memo from E. Budd to D. Pilitt dated December 5, 1985 entitled "Addendum to Lindane Registration Standard. Requirement for Additional Toxicity Studies") the registrants of lindane (C.I.E.L., Centre International d'Etudes du Lindane) have submitted a 13 week dermal toxicity study with lindane with rabbits. This study was requested partly in order to clarify the species selectivity of certain kidney effects and to assess the blood for toxicity to the formed elements. Thus, the study protocol included special assessments for kidney function and pathology as well as bone marrow myelograms.

The study was reviewed and the following comments apply.

Toxicology Branch Comments

1. The study was classified as CORE GUIDELINE. The data requirement for a 90-day subchronic dermal toxicity study (82-3) in rabbits has been satisfied.

2. A slight but statistically significant ($p < 0.01$) increase (4-6%) in relative kidney weight in the high dose (320/350/400 mg/kg/day) male group in this rabbit study was noted. Female high dose group kidney weights were also increased (5.4 to 6%, $p < 0.001$). These apparent weight changes were not accompanied by any histological changes in either sex.

Kidney weight gain in male rats is one of the characteristic symptoms of the lindane induced kidney pathology being investigated (refer to J. Doherty review dated April 3, 1989, cover memo dated May 18, 1989). It might be possible but TB-I considers it unlikely that the high dose group is a threshold for effects of lindane in the male rabbit kidney and the only manifestation of the threshold effect in the rabbit is increased weight. For comparative purposes, the male rat had slight increases in kidney weight (+5%, ns) at 10 mg/kg/day but this level was also considered the threshold level for histological changes. Maximum kidney weight increase in the male rat (+13.8%, relative weight increase, $p < 0.001$) was at 400 mg/kg/day. Thus, in the rat, the kidney weight change is associated with the pathological change unlike the situation in

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the rabbit.

Based on the absence of histological changes in the male rabbit kidney, TB-I has decided that this study demonstrates that the rabbit kidney is not sensitive to lindane with regard to developing a specific kidney pathology at dose levels at or below 320/350/400 mg/kg/day applied dermally.

3. At week 7 (interim sacrifice) there were some apparent decreases in RBC count, packed cell volume and hemoglobin but none of these reached statistical significance. At recovery, all RBC cell parameters were considered comparable to the control. At week 13, however, there were statistical decreases in hemoglobin (-7%, $p < 0.01$), RBC count (-8.6%, $p < 0.01$) and PCV (-5.7%, $p < 0.01$). These decreases might suggest an anemia. Bone marrow analysis, however, did not indicate any effects on the formation of blood elements at any time. Thus. TB-I concludes that the data do not demonstrate that lindane induced a defined anemia in this study. The decrease in RBC parameters at week 13 is noted, however and comparisions should be made with other studies which investigate blood parameters.

4. The NOEL for this study was set at 10 mg/kg/day. The LEL was set at 60 mg/kg day based on increased incidents of liver "centrilobular hypertrophy" which may represent an adaptation/ metabolic response to lindane.

Adrenal weight (both absolute and relative) was also elevated starting at 60 mg/kg/day for males (~60% $p < 0.05$)) and increasing to 50% in the high dose group ($p < 0.001$) but the increases in the weight of this organ were not accompanied by histological changes. There were no special adrenal function tests or hormone studies to assess the function of this organ. The general pattern of observed toxicity in this study did not indicate that adrenal function was affected.

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Reviewed by: John Doherty 4/16/91
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DATA EVALUATION REPORT

STUDY TYPE: 82-3. 90 day dermal toxicity - rabbits

MRID NO.: 414276-01 TOX. CHEM. NO.: 527

TEST MATERIAL: Lindane (99.5% gamma-hexachlorocyclohexane) from lots DA433 batches 1 and 2. Also called HD 300/86-580.

TEST ANIMALS: New Zealand White Rabbits obtained from Hazleton Research Products, Inc. Pennsylvania, USA.

STUDY NUMBER(S): 6164-580/6

SPONSOR: Centre International d'Etudes du Lindane (CIEL)

TESTING FACILITY: Hazleton, Inc. North Yorkshire, England

TITLE OF REPORT: "Lindane: 13 week dermal toxicity study (with interim kill and recovery period) in the rabbit".

AUTHOR(S): D. Brown

REPORT ISSUED: February 22, 1990

CONCLUSIONS:

NOEL = 10 mg/kg/day. LEL = 60 mg/kg/day: Liver pathology (centrilobular hypertrophy, both sexes) and increased adrenal weight (males). At 320/350/400 mg/kg/day: tremors and deaths; body weight decreases (males and females); liver weight (males and females); RBC parameters (< 9% for RBC count, PCV and hemoglobin, males); alkaline phosphatase and/or glutamic pyruvate transaminase (both sexes); kidney weight, increased 4-6% for both sexes. No evidence that lindane caused histopathological changes in the male kidney or was toxic to the formed elements of the blood was apparent.

Dose levels tested 0, 10, 60, and 320/350/400 mg/kg/day dermally.

Classification: CORE-GUIDELINE. This study satisfies the requirement for a subchronic 90-day dermal toxicity study (82-3).

Quality Assurance Statement: A statement signed by Pamela R. Cooper, Quality Assurance Manager attested that 20 audits or

inspections were made and 16 reports prepared. The Quality Assurance page did not indicate deficiencies in the conduct or reporting of the study.

REVIEW

The basic design of this study consisted of four groups of 40 male and 40 female rabbits which were dosed with either 0, 10, 60 or 400 (initial dose) mg/kg/day of lindane. The protocol included an interim sacrifice group of ten rabbits of each sex per dose group which were sacrificed after 6 weeks of dosing. A Main phase which consisted of 20 rabbits of each sex dosed for 13 weeks and a recovery group of 10 rabbits of each sex in each group which were allowed 6 weeks to recover from any reactions following dosing for 13 weeks. The high dose group (originally 400 mg/kg/day) proved to be too toxic to the rabbits and was reduced to 350 mg/kg/day from week nine and to 320 mg/kg/day from week 11. All forty rabbits for each dose level were not dosed simultaneously. The rabbits in the interim sacrifice group and recovery group were initiated in October of 1988 and sacrificed in November (interim) and February, 1989 (recovery). The animals in the main phase were initiated in January, 1989 and sacrificed in April. This staggered procedure was considered necessary because of space limitations and time factors involved in properly treating animals on a daily basis.

The test material was applied to the rabbits dissolved or suspended in 5% aqueous carboxymethyl cellulose. The test solution was applied at the rate of 2 ml/kg by means of a syringe to a patch and the patch was then applied to the shaved (at least once a week) back of the rabbit to cover an area approximately 10% of the body surface and kept in place by means of a canvas jacket. This patch consisted of a "thin layer of porous gauze, heat welded to an aluminum foil backing". The use of this gauze was previously discussed with TB-I. The test patch was applied to the same area at each application and was said to be kept in place for at least six hours per day. After six hours, the patch was removed, the amount of test article estimated and the area swabbed and washed with infant soap. Dosing was five days per week (Monday through Friday). Dosing was often suspended until the animals recovered for the high dose group when these animals showed signs of tremors.

Proof of Absorption.

A separate report Appendix 12, entitled "Proof of Absorption" authored by I. Smith and L.J. Phillips and dated February 22, 1990 (pages 536 to 790 or 254 pages) provided a comprehensive assessment of the proof of absorption of lindane following dermal application in this study.

According to the report, prior to starting the analyses, the

methods for quantifying lindane in blood, adipose tissue, kidney, liver and brain were validated. The limits of detection for these tissues were as follows: plasma 5 ng/ml, kidney, liver and brain 0.01 ug/gm, and adipose tissue 2 ug/gm. Samples of the blood and tissues were obtained from each dose group and the control at necropsy at weeks 7, 14 and 20 and were assessed by gas chromatography for lindane content.

Copies of Tables 12.6.1, 12.6.6 and 12.6.7 xeroxed from the study report are attached to illustrate the results of these analyses. In summary, the content of lindane in the control group and in all animals at week 20 was below the limit of detection for all samplings. The individual tissue and plasma results are discussed as follows for weeks 7 and 14. Note: there were 10, 20 and 10 rabbits of each sex originally available for sampling for the 7, 14, and 20 week assays.

Plasma: Mean plasma levels reached as high as 500 (±204) ng/ml in the high dose group females at week 14. Females had the higher levels for most all dose groups and times. Due to the large standard deviations involved, it is difficult to determine if the plasma level is actually higher at week 14 than at week 7. As would be expected, higher levels were found in the groups treated with the higher dose levels of lindane.

Adipose tissue: Lindane concentrations were highest in adipose tissue reaching levels as high as 277 (± 89) ug/gm at week 14 in females. Male levels were probably the same or a little less than female levels (large standard deviations were noted and no statistical analysis was presented, see tables). Evidence of accumulation between week 7 and 14 was not apparent for the low dose group but was apparent for the mid and high dose groups.

Liver: Liver had the second highest levels reaching 7.38 (± 5.6) ug/gm for males at week 14. Since the levels at week 14 were similar to those at week 7 for all dose groups, there was not much accumulation.

Kidney: The kidney is regarded as a target organ for lindane in some species. Lindane levels reached as high as 7.12 (± 2.201) ug/gm in female high dose group, males were slightly lower. Some evidence of accumulation was apparent in the mid and high dose groups but not in the low dose group (see tables).

Brain: The lowest dose levels were reported in the brain with the maximum being 6.13 (± 2.403) ug/gm in females at week 14. Evidence of accumulation was apparent for the mid and high dose groups but also possible for the low dose females which had 0.14 (± 0.021) ug/gm at week 7 and 0.024 (± 0.095) ug/gm at week 14.

Results

A. Clinical signs. The clinical signs reported included tremors and convulsions. These were noted throughout the study in the high dose group (males after day 16 and females after day 19). Up to three animals in the high dose group displayed these symptoms at any one observation day. Only a single female in the mid dose group (on day 50 only) displayed these symptoms. Compound related reactions at the site of application were not reported.

NOEL (clinical signs) = 60 mg/kg/day. LEL = 320/350/400 mg/kg/day: Tremors. The single incident of tremors in the mid dose group female is not considered sufficient to justify assigning this level as an effect level.

B. Survival. In the high dose group 17 males (1 interim, 10 main and 6 recovery group) and 8 females (5 main and 3 recovery group) died (only three that died were replaced by new animals). Deaths (or removal in moribund condition) came after week 5. The cause of death was believed to be related to the neurotoxicity of lindane since these animals showed tremors and convulsions. None of the rabbits in the control, low or mid dose groups died.

NOEL (survival) = 60 mg/kg/day. LEL = 320/350/400 mg/kg/day. Deaths in males and females.

C. Body Weight and Food Consumption.

In the main study (considered to best represent the body weight effects because each group had more animals), the high dose group males started to loose weight after the first week of dosing and were from 3 to 7% lower in weight until week 13. The females also started to loose weight at week 1 and were from 3 to 10% lower in weight until week 13. The low and mid dose body weights were essentially equivalent to the controls.

During the recovery phase the weight decrement in the high dose males remained 3 to 8% below the controls. At week 19, the male group was still 8% less than the controls. The females, however, showed signs of regaining the lost weight, during the recovery period the females were 1 to 3% lower in weight and at week 19 were only 1% lower.

Note: The study report did not present a statistical analysis of the body weight data with regard to making comparisions with the control groups. The standard deviations were, however, usually < 10% of the means.

In general, the high dose group males and females ate less food than the controls consistent and roughly parallel with the decrease in body weight. During recovery, the males apparently ate more but did not regain their lost weight.

CONCLUSION (body weight gain): NOEL = 60 mg/kg/day. LEL = 320/350/400 mg/kg/day: Decrease in body weight in males and females.

D. Ophthalmoscopy.

The report states that there "was no pattern of ophthalmoscopic change to suggest an effect of treatment". The data were not tabulated. According to the protocol the animals (all available) were examined at predosing, at week 6 (interim kill only), at week 12 (main study) and at weeks 13 and 19 (recovery groups).

Note: For clinical chemistry and hematology, blood samples were taken at necropsy (interim, main study and recovery) and when possible from moribund rats from the dorsal aorta or vena cava while the rat was under anesthesia. At week 13 blood was sampled from the jugular vein from the recovery animals. Baseline values were obtained prior to the start of the study.

E. Clinical Chemistry. The following parameters were investigated: aspartate aminotransferase/glutamate pyruvate transferase, alkaline phosphatase, lactate dehydrogenase, gamma glutamyl transferase (GGT), Na+, Cl-, inorganic phosphorous, urea, creatinine, albumin, total cholesterol, K+, Ca++. glucose, total bilirubin, total protein, albumin globulin ratio. Alkaline phosphatase and GGT activities were analyzed by the Kruskal Wallis and WIlcoxson Rank Sum tests at weeks 7 and 13. The statistical test used at other intervals was not indicated.

Note: The blood samples from the recovery group were stated in the report as being unsuitable for analysis because of marked hemolysis apparently resulting from an incorrect withdrawal technique.

The following deviations were noted.

Alkaline phosphatase: Was increased at the interim sacrifice for high dose group females (+34%, $p < 0.05$), for the main study for males (+44%, $p < 0.001$) and females (+53%, $p < 0.001$) in the high dose group.

GGT: Was increased in the main study for females in the high dose group (+38%, $p < 0.001$).

NOEL (Clinical Chemistry): 60 mg/kg.day. LEL = 320/350/400 mg/kg/day: increases in alkaline phosphatase and glutamate pyruvate transferase in females and/or males.

Note: The increase in these enzymes suggest organ damage particularly liver damage but other facotrs may also result in increases in these enzymes.

F. Hematology. The parameters investigated were hemoglobin, mean cell volume, red blood cell count (including mean cell hemoglobin and hemoglobin concentration and packed cell volume), total and differential white blood cell count and platelet count. Statistics were by analysis of variance and t-test and the Kruskal Wallis and Wilcoxson Rank Sum tests.

Interim Sacrifice: Among the males, there were apparent decreases in high dose group RBC count (-1.5%, ns), PCV (-6.4%, ns) and hemoglobin (-3.2%, ns). Decreases reached statistical significance for MCV for the mid (-5%, $p < 0.01$) and high (-5%, $p < 0.05$). Absolute and proportional neutrophil counts were reported as being elevated but not significantly. Since none of these parameters reached statistical significance, TB-I does not consider that lindane effects the blood elements at this time interval.

Main Sacrifice: Among the males, there were apparent decreases in the high dose group for hemoglobin (-7%, $p < 0.01$), RBC count (-8.6%, $p < 0.01$), and PCV (-5.7% $p < 0.05$). MCV was slightly higher than the control for this group (+2.6%, ns). The high dose male (-9.3%, ns) and female (-12%, ns) total white blood cell count was lower than the control.

Recovery Sacrifice: Male red blood cell parameters were comparable to the controls. The high dose group males had total WBC counts (-37%, $p < 0.01$) and absolute lymphocytes counts (-37%, $p < 0.05$) lower than the controls. The low dose group also had lower lymphocyte counts (-22%, $p < 0.05$). The WBC data did not show a consistent pattern of possible effects of lindane.

Myelograms. The study author maintains that the myelograms showed no consistent pattern of change to indicate an effect of treatment. The myelogram taken from a sample of bone marrow reported tabulations for the following parameters: proerythrocytes, early intermediate and late erythroblasts, megaloblast, promyelocyte, myelocyte, metamyelocyte, neutrophil, eosinophil, basophil, lymphocyte, monocyte, megakaryocyte, plasma cell, reticulum cell, total erythropoietic cells, total granulopoietic cells and myeloid/erythroid ratio.

Inspection of the data present confirm that there were no consistent patterns to indicate a toxic response to lindane. The

clinical hematology findings above were not corroborated by changes in blood element formation.

NOEL (Hematology): NOEL = 60 mg/kg/day. LEL = 320/350/400 mg/kg/day: decreases in RBC parameters (hemoglobin, PCV, and RBC counts) and WBC in males at week 13.

NOTE: The apparent changes in WBC elements in males at lower doses were not considered to be of sufficient consistency or magnitude to be considered an effect of the test material.

G. Urinalysis

Special Kidney Function Test (Water Loading Study). In this special study which was performed just prior to necropsy, the rabbits were "water-loaded" (20 ml/kg) by gavage and then deprived of food and water for approximately 18 hours during collection. Urine remaining in the bladder was collected by means of a hypodermic syringe. For comparison urine was collected from some "sporadic animals when possible. Baseline urinalysis was obtained before the start of the study by collecting the urine from the rats overnight and at necropsy with an hypodermic syringe.

The following parameters were investigated: appearance, volume, specific gravity, protein*, ketones*, blood*, reducing substances*, color, pH*, total bilirubin*, urobilinogen* microscopy of deposits, urea, and creatinine (*semi quantitative determination by BM-Test-7 sticks or equivalent testing methodology). Statistics were by analysis of variance and t-test and the Kruskal Wallis and Wilcoxson Rank Sum tests.

The study report conclusion is that "There was no consistent pattern of difference to suggest an effect of treatment".

NOEL (Urinalysis): NOEL = 320/350/400 mg/kg/day (HDT).

H. Organ Weights. Data were presented which indicated that the weights (absolute and relative) for the adrenals, kidneys, ovaries, testis, brain, liver, spleen and thymus were weighted for the 6 week interim kill, main study and recovery phase. Separate weights were reported for each (left and right) of the paired organs. Organ weights for animals dying during the study were not reported. The data were analyzed by ANOVA and t-test.

a. Kidney. Some deviations from the control were noted as follows:

Interim (6 week sacrifice): differences not statistically significant.

Male absolute weights were reduced -3 to -8%, with both high dose (left and right) being 8% less than the control. A dose response was not recognized and the low dose group was -4 and -7% less for the left and right kidneys.

Male relative weights were also reduced -3 to -7%, with both high dose (left and right) being 5% less than the control. A dose response was not recognized and the low dose group was -3 and -7% less for the left and right kidneys.

Main (13 week sacrifice): Statistically significant differences in the relative weights for male and female kidneys were noted as indicated in the following table.

Kidney Weights (S.D.)

Dose Group		Male Absolute/Relative	Female Absolute/Relative
Control	L	7.795(0.980)/.2258(.0201)	7.692(0.562)/.2073(.0178)
	R	7.819(0.938)/.2266(.0190)	7.661(0.629)/.2064(.0181)
10 mg/kg	L	7.713(1.323)/.2186(.0327)	7.500(0.949)/.2023(.0245)
	R	7.735(1.182)/.2194(.0296)	7.508(0.841)/.2026(.0221)
60 mg/kg	L	7.755(0.944)/.2229(.0228)	7.538(0.739)/.2043(.0168)
	R	7.852(0.754)/.2261(.0196)	7.402(0.637)/.2006(.0122)
"400"mg/kg	L	8.286(0.892)/.2580(.0145)**	8.151(1.071)/.2399(.0273)***
	R	8.146(0.732)/.2541(.0135)**	8.078(1.041)/.2379(.0278)***

** $p < 0.01$, *** $p < 0.001$ Study report statistics by ANOVA and t-test.

The above table shows that both the male high dose group (4 to 6%) and female (5.4 to 6%) relative kidney weight is higher than the controls.

The increase in kidney weight in the high dose group males is one characteristic of the kidney effects of lindane in the male rats. In rats, a dose level of 400 mg/kg/day resulted in a 13& increase in relative weight. Relative weights at the 6-7 week interval in rats were elevated slightly (refer to DER for the rat dermal toxicity study by J. Doherty dated April 3, 1989), but in rabbits they were decreased slightly.

Recovery: All dose groups were reasonably close to the control groups.

b. Liver. The following increases in liver weight were noted:

Interim:

Female, high dose group: Absolute +27.24%***; relative +30.53%***.

Main:

Female, high dose group: Absolute +27.01%***; relative +44.98%***

Male, high dose group: Absolute no change; relative +36.77%***.

Recovery:

Female, high dose group: Absolute 13.00%, relative 17.31%, Not significant.

*** p < 0.001, ANOVA and t-test study report statistics.

c. Adrenals.

Interim: No statistical differences reported but absolute/relative male (14-16%/19%) and female (5-7%/7-11%) weight increases were noted in the high dose group.

Main: Statistical significance was attained as follows.

Adrenal Weight (SD)

Dose Group		Male Absolute/Relative	Female Absolute/Relative
Control	L	.200(.053)/.0058(.0015)	.177(,064)/.0048(.0017)
	R	.175(.041)/.0051(.0012)	.159(.055)/.0043(.0014)
10 mg/kg	L	.234(.068)/.0066(.0020)	.188(.059)/.0051(.0016)
	R	.215(.049)/.0061(.0014)	.169(.051)/.0045(.0013)
60 mg/kg	L	.239(.057)*/.0069(.0016)*	.195(.056)/.0053(.0014)
	R	.216(.056)*/.0062(.0016)*	.171(.045)/.0046(.0012)
"400" mg/kg	L	.281(.114)**/.0087(.0032)***	.236(.084)**/.0070(.0025)***
	R	.256(.099)**/.0080(.0028)***	.213(.066)**/.0063(.0019)***

* < 0.05, ** p < 0.01, *** p < 0.001. Study report statistics by ANOVA and t-test.

The above table illustrates that the adrenal weights were 19.5 to 23.4% increased (absolute) and 19.0 to 21.6% increased (relative) for the mid dose group males and 40.5 to 46.3 (absolute) and 50.0 to 56.9 (relative) for the high dose

group males. Only the high dose group females were increased (33-34%, absolute and 46 to 46% relative).

Recovery: Adrenal weights were equivalent to the controls for both absolute and relative determinations.

d. Thymus

The high dose thymus weight (absolute and/or relative) was reported as being lower for both males and females for both the interim and main aspects of the study but not for the recovery phase. Statistical significance was not attained. Large standard deviations affected the statistical analysis.

NOEL (Organ Weight): NOEL = 10 mg/kg/day. LEL = 60 mg/kg/day: adrenal weight increase (~20%, males). At 320/350/400 mg/kg: adrenal weight (~50% males and females); liver weight both males and females (>27%). Kidney (males increased, females decreased).

Note: Thymus weight gain data did not reach statistical significance.

I. Pathology. A separate pathology report (Appendix 16) was prepared. The principal author was D. Brown and the report is dated February 22, 1990. Dr. Brown asserts that the only treatment related histological findings were "centrilobular hypertrophy" in the groups dosed with 60 and 320/350/400 mg/kg/day.

Only the control and high dose groups were examined histologically for most organs. The liver, three samples of each kidney, sternal and femoral marrow and gross lesions for the low and mid dose groups were also analyzed.

a. Skin. Lindane was applied to the skin of the animals and in this type of study the site of application often develops reactions to the test material.

There were no dose related increases in dermal lesions noted.

b. Kidney. This study was designed especially to investigate the possibility that lindane might affect the kidney and produce histopathological lesions in this organ.

There were no dose related incidents of lesions in the kidneys as observed at gross necropsy. The conditions described as irregular surface, pale and adhesion were occasional in occurrence. All were singular instances except for the mid dose female group which had 2 incidents of irregular surface at the 6

week interval. Irregular surface was not reported in females at either the ~~13~~ week or recovery sacrifices.

The following table depicts the results of the histopathology of the kidney for the main study or rats sacrificed at week 13.

Lesion	Sex	Control	low	mid	high
Number examined	M/F	20/20	20/20	20/20	10/15
Focal Nephropathy	M	19	20	19	10
	F	19	19	20	15
Glomelero-Nephropathy	M	1	0	1	0
	F	0	3	0	0
Tubular Dilation	M	0	0	0	0
	F	0	0	1	0
Mineralization	M	17	18	16	8
	F	14	9	11	9
Fibrosis	M	0	0	0	0
	F	2	0	0	0
Hyaline Droplets	M	3	1	3	3
	F	5	8	5	3
Pigment	M	5	4	0	1
	F	7	11	4	6
Interstitial nephritis	M*	1	5	5	5
	F*	2	2	4	5
	M	0	1	1	0
	F	0	0	0	1

*These data are from the 6 week interim sacrifice and are for 10 rabbits of each sex except for the high dose male group which had 9.

In addition to the above lesion types there were also present cysts and leucocyte foci and pyelitis but htese were occasional findings and did not demonstrate a dose relationship.

The lesions characteristic of nephropathology of lindane in rats are tubular degeneration (none reported at any interval in the rabbit study), hyaline droplets, basophilic

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tubules (regenerative and atrophic) and swelling. There were no dose related increases in these lesion types in the main phase (as shown above) or in the interim or recovery phases.

c. Liver. The liver weight was increased for both sexes and serum enzyme activity was elevated suggesting an effect of the test material in this organ. The liver has been demonstrated to be a target organ for lindane in other studies by other (i.e. oral and dermal) routes of exposure.

The incidence of centrilobular hypertrophy was elevated at the interim, main and recovery sacrifice times as indicated in the following table.

Interval	Sex	Control	10 mg/kg	60 mg/kg	350/400 mg/kg
Interim	M	0/10	0/10	2/10(20%)	9/9(100%)
	F	0/10	0/10	3/10(30%)	9/10(90%)
Main	M	0/20	0/20	4/20(20%)	8/10(80%)
	F	0/20	0/20	5/20(25%)	11/15(73%)
Recovery	M	0/10	0/10	3/10(30%)	2/4(50%)
	F	0/10	0/10	4/10(40%)	2/7(29%)

Data are incidents/number of animals observed.

No statistical analysis was presented but it is very clear that at 60 mg/kg/day and above more that 20% of the rats have the condition of centrilobular hypertrophy in the liver. None of the rats dosed with either the control or 10 mg/kg/day have this condition.

The pathology report author also presented summary tables which indicated that the degree of grading (scale 0, 1,2 or 3) increased with the dose levels (i.e more grade 3 conditions were noted in the highest dose level).

d. Adrenals. Adrenal weights were elevated for both the males and females. Only the control and high dose groups were examined. Singular incidence of several different lesion types were reported without indication of a dose response. In addition there were 2 incidents of "accessory nodule" in the female high dose group and none in control.

The changes in adrenal weight were not accompanied by pathological changes. There were no adrenal function tests run to further assess the status of this organ.

e. Thymus. There were some indications that thymus weights were

affected. Occasional incidents of "ectopic parathyroid" were reported without regard to a dose response.

NOEL (pathology) = 10 mg/kg/day. LEL = 60 mg/kg/day: liver (centrilobular hypertrophy).

CONCLUSION (study). This study is CORE GUIDELINE. The data do not indicate that the rabbit kidney is affected with histological changes following dermal exposure (a stated objective of the study). No indication that lindane was toxic to the formed elements of the blood was apparent.

The following "one liner" applies to this study:

NOEL = 10 mg/kg/day. LEL = 60 mg/kg/day: Liver pathology (centrilobular hypertrophy, both sexes) and increased adrenal weight (males). At 320/350/400 mg/kg/day: tremors and deaths; body weight decreases (males and females); liver weight (males and females); RBC parameters (< 9% for RBC count, PCV and hemoglobin, males); alkaline phosphatase and/or glutamic pyruvate transaminase (both sexes); kidney weight, increased 4-6% for both sexes. No evidence that lindane caused histopathological changes in the male kidney or was toxic to the formed elements of the blood was apparent.

Dose levels tested 0, 10, 60, and 320/350/400 mg/kg/day dermally.

TABLE 12.6.1

Rabbit plasma study sample analyses: summary of HD 300/86-580 concentration (ng/ml)

Group number		Week 7 Male	Week 7 Female	Week 14 Male	Week 14 Female	Week 20 Male	Week 20 Female
1	Mean	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
	S.D.	-	-	-	-	-	-
2	Mean	11.5	11.2	15.8	21.6	<5.0	<5.0
	S.D.	5.94	3.86	4.94	11.87	-	-
3	Mean	42.5	46.5	76.7	98.6	<5.0	<5.0
	S.D.	31.91	23.80	41.40	41.63	-	-
4	Mean	238	358	418	500	<5.0	<5.0
	S.D.	155.1	209.8	141.2	203.6	-	-

TABLE 12.6.7

Rabbit tissues study sample analyses: summary of HD 300/86-580 concentrations at week 14 (µg/g)

Group number		Kidney		Liver		Adipose		Brain	
		Male	Female	Male	Female	Male	Female	Male	Female
1	Mean	<0.10	<0.10	-	<0.10	<2.00	<2.00	<0.10	<0.10
	S.D.	-	-	-	-	-	-	-	-
2	Mean	0.20	0.21	0.61	0.47	11.0	10.5	0.17	0.24
	S.D.	0.079	0.061	0.331	0.217	2.58	2.83	0.056	0.095
3	Mean	1.23	1.16	1.43	0.72	60.0	62.3	1.06	1.10
	S.D.	0.566	0.499	1.05	0.487	22.90	15.10	0.451	0.352
4	Mean	6.74	7.12	7.38	7.32	271	277	5.87	6.13
	S.D.	2.723	2.201	5.595	3.178	78.6	88.6	2.314	2.403

TABLE 12.6.6

Rabbit tissues study sample analyses: summary of HD 300/86-580 concentrations at week 7 (µg/g)

Group number		Kidney		Liver		Adipose		Brain	
		Male	Female	Male	Female	Male	Female	Male	Female
1	Mean	<0.10	<0.10	<0.10	<0.10	<2.00	<2.00	<0.10	<0.10
	S.D.	-	-	-	-	-	-	-	-
2	Mean	0.17	0.19	0.47	0.51	8.67	11.8	0.15	0.14
	S.D.	0.042	0.055	0.255	0.211	3.010	6.41	0.015	0.021
3	Mean	0.59	0.76	1.66	1.26	28.3	38.4	0.45	0.56
	S.D.	0.308	0.287	0.921	0.447	10.87	10.26	0.194	0.115
4	Mean	3.20	3.87	6.61	5.84	153	196	2.28	3.19
	S.D.	0.593	1.253	1.482	2.321	30.9	66.9	0.590	1.137

Inhalation
90 day dermal - rabbits
Hazleton (UK) 6164-586/6
MRID # 414276-01
Feb 22, 1992

82-3 Repeated Dose Dermal Toxicity (90-day) in the Rat, Rabbit or Guinea Pig

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 10 animals/sex/group (3 test groups and control group).
3. ✓ Dosing duration at least 6 hour/day daily for 90 days or 5 days/week for 13 weeks.
4. ✓ Application site at least 10% of body surface area.
5. ✓ Doses tested include signs of toxicity at high dose, no or minimal dermal irritation, minimal lethality or a limit dose (1000mg/kg) if nontoxic.
6. • ✓ Doses tested include a NOEL.
7. ✓ Individual daily observations.
8. ✓ Individual body weights.
9. ✓ Individual or cage food consumption.
10. • ✓ Opthalmoscopic examination (at least pretest and at term) control and high dose.
11. ✓ Clinical pathology data of 12 & 13 in all animals at termination.
12. ✓ Hematology.
    - ✓ Erythrocyte count
    - ✓ Hemoglobin
    - ✓ Hematocrit
    - ✓ Leucocyte count
    - • ✓ Differential count
    - ✓ Platelet count (or clotting measure)

    mean cell volume
    " " hemoglobin
    " packed cell volume
13. ✓ Clinical chemistry.
    - ✓ Alkaline phosphatase
    - ✓ Aspartate aminotransferase
    - • ✓ Creatinine kinase
    - ✓ Lactic dehydrogenase
    - ✓ Glucose
    - ✓ Bilirubin
    - ✓ Cholesterol
    - • ✓ Creatinine
    - ✓ Total Protein
    - ✓ Albumin
    - ✓ Urea
    - ✓ Inorganic phosphate
    - ✓ Calcium
    - • ✓ Potassium
    - ✓ Sodium
    - • ✓ Chloride
14. • ✓ Urinalysis, only when indicated by expected or observed activity. As scheduled in 11.
    - ✓ Blood
    - ✓ Protein
    - ✓ Ketone bodies
    - ✓ Appearance
    - ✓ Glucose
    - ✓ Total bilirubin
    - • ✓ Urobilirubin
    - ✓ Sediment
    - ✓ Specific gravity (osmolality)
    - • ✓ Volume

    + water loading data
15. ✓ Individual necropsy of all animals.
16. ✓ 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
    - ✓ eyes
    - ✓ jejunum
    - ✓ bone marrow
    - ✓ peripheral nerve sciatic
    - ✓ kidneys‡ ✓

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

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

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