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

MEMORANDUM

JUN 17 1983

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: George LaRocca, PM#15
Registration Division (TS-767)

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769)
and
William L. Burnam, Chief
Toxicology Branch/HED (TS-769)

Budd 6/16/83

WLB 6-16 83

SUBJECT: Lindane: Subchronic Oral Dosing (Rat) ~~Study and~~ a Metabolic Study (Mouse). Tox. Chem. No. 527

This submission consists of two studies entitled:

1. 3-Months Toxicity Study In Rats With LINDANE. Project No. 005220, dated 2/3/83. Accession Numbers: 250340-250342.

2. Covalent Binding To Mouse Liver DNA As Inconclusive Mechanism Of Carcinogen Action of Hexachlorocyclohexane Isomers. (No study number). Study date: 4/15/83. Accession No.: 250339

NOEL in the 3-month feeding study is 4 ppm (0.3 mg/kg/day; male and female rats) and is based on systemic effects. The LEL is 20 ppm or 1.55 mg/kg of body weight/day for the male rats and 1.67 mg/kg of body weight/day for the female rats. Permanent or long-lasting (could not be reversed in 6 weeks) kidney damage was observed at this level in both sexes, but especially in the males. Levels of lindane tested: 0, 0.2, 0.8, 4, 20 or 100 ppm.

With regard to the metabolic study, it was concluded that the alpha and gamma hexachlorocyclohexane isomers were non-mutagenic liver carcinogens, meaning that they did not bind to DNA with any degree of significance. The gamma isomer is, of course, lindane.

A detailed evaluation of the 3-month rat feeding study and a general evaluation of the metabolic study with male mice are submitted with this memorandum.

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TS-769:th:TOX/HED:KKLocke:6-15-83:card misc.#29

STUDY TYPE: Subchronic Oral Dosing (90 days; rat).

STUDY TITLE: 3-Months Toxicity Study In Rats With LINDANE. Project No. 005220.

ACCESSION NO.: 250340-250342 TOX. CHEM. NO.: 527

RECORD NO.: 98077

SPONSOR: Centre International d'Etudes du Lindane (C.I.E.L.), Bruxelles

TESTING LABORATORY: RCC, Research and Consulting Company Ltd., 4452 Itingen, Switzerland.

DATE OF FINAL REPORT: February 3, 1983

DURATION OF STUDY: 12/7/81-4/19/82 (includes one week of an acclimation or pretest period and a 6-week recovery period).

TEST MATERIAL: Lindane (1α, 2α, 3β, 4α, 5α, 6β-hexachlorocyclohexane; 99.85% pure; batch number: 81044/166).

PROTOCOL:

Wistar KFM-Han (outbred) SPF rats, 20 of each sex/level, were fed diets containing the following levels of lindane (ppm): 0 (Group 1), 0.2 (Group 2), 0.8 (Group 3), 4 (Group 4), 20 (Group 5) and 100 (Group 6). The feeding was continued for 12 weeks, at which time 15 rats from each group (those with the lowest ID numbers) and sex were sacrificed (by exsanguination in ether narcosis). The remaining 5 male and 5 female rats from the lindane - fed Groups 2, 3, 4, 5 and 6 were subsequently placed on a control diet for 6 weeks (recovery period) and then were sacrificed. The remaining 5 male and 5 female rats from the control Group 1 were also continued on their diets for 6 more weeks before they were sacrificed. The animals were allocated to the different groups by means of a random algorithm.

The rats were obtained from KFM Kleintier farm Madoerin AG (4414 Fuellinsdorf/Switzerland) and were 8 weeks old upon arrival to the testing laboratory. Males weighed 202-260 g and females 165-202 g. Dosing began after one week of the acclimatization (or pretest) period and examination by a veterinarian. The animals were housed in groups of 5, in macrolon cages with wire mesh tops and granulated soft wood bedding. Each rat was identified by cage number and toe clipping. The temperature of the room was 22 ± 2°C and the relative humidity was 55 ± 10%.

The diet used was Kliba 343 Rat/Mouse Maintenance Diet, purchased as pellets. Lindane (a white powder) was added to the ground pellets and the mixture was then repelletized. The diets were prepared every 4 weeks and were stored in paperbags at room temperature, in a separate room. Both water and Kilba 343 pellets were analyzed for contamination. The diets were also analyzed for lindane content, stability and homogeneity. Food and water were allowed without restriction.

The following parameters were examined in this study:

1. Observation for Toxic Signs and Mortality - Observations were made every morning and afternoon throughout the study. A detailed clinical examination, including palpation, of all animals was performed every week.

2. Body Weight - All animals were weighed weekly, from the experimental week 1 (acclimation or pretest week) through the experimental week 19 (termination of the recovery period).

3. Food Consumption - Food consumption was measured every 7 days during the pretest, dosing and recovery periods.

4. Eye Examination - This examination was performed on 10 rats/group/sex (those with the highest ID numbers) prior to dosing and at the completion of dosing, and on all rats (or 5/group/sex) at the termination of the recovery period. Eyes were examined with an ophthalmoscope.

5. Hematology, Clinical Biochemistry and Urinalysis - Except when specified otherwise, these determinations were performed on 10 male and 10 female rats (those with the lowest ID numbers) during the study weeks 1 (pretest), 6 and 13 or dosing weeks 0, 5 and 12, respectively. All of the rats assigned to the recovery groups (5 males and 5 females/dose) were also subjected to these tests during the first and after the last (6th) week of the recovery period. Procedures used in various determinations were not referenced or detailed, but their principles were stated and the analytical instruments used were identified. (Example: Total protein was determined by the biuret procedure, using Greiner Selective Analyzer G-300).

Blood samples were obtained by an orbital sinus puncture and always between 7:00 and 9:00 a.m., following an overnight (18 hours) deprivation of food. Urine was collected by placing the rats individually, without food, in the metabolic cages for 18 hours (overnight). However, the rats were also inadvertently deprived of water during the study weeks 13 (end of dosing) and 19 (end of recovery period). Brain and liver tissue, used for the determinations of certain enzymes, were weighed at the time of sacrifice, frozen in liquid nitrogen and the stored at - 20°C until analyzed. The following determinations were performed:

Hematology

- °Erythrocyte count; hemoglobin concentration and hematocrit; mean corpuscular volume (MCV), hemoglobin (MCH) and hemoglobin concentration (MCHC).
- °Total and differential leukocyte counts, reticulocyte and platelet counts; and thromboplastin and partial thromboplastin times.

Clinical Biochemistry

- °Glucose, urea, creatinine, total cholesterol and total bilirubin content.
- °Aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and alkaline phosphatase activities.
- °Total protein and protein gel electrophoresis (determination of albumin, 3 globulin fractions and albumin/globulin ratios).
- °Sodium and potassium content.
- °Plasma butyryl- and erythrocyte acetylcholinesterase activities.
- °Brain acetylcholinesterase activity (determined for 5 male and 5 female rats/group at the termination of dosing only).
- °Plasma carboxylesterase (aliesterase) activity.
- °Liver tissue homogenate carboxylesterase (aliesterase) and aminopyrine N-demethylase activities, and cytochrome P-450 content. (All were determined for 10 male and 10 female rats/group at the termination of dosing, and for all rats at the completion of the recovery period)..

Urinalysis

°Volume (ml/18 hours) and color (visual evaluation).

°Specific gravity.

°Protein, pH, glucose, ketones, bilirubin, blood, urobilirubin and nitrites, all determined by means of a Reagent-Test-Strip (Ames N-Multistix).

°Urine sediment (centrifugation of specimen at 1000 rpm for 10 minutes).

6. Levels of Lindane in Tissues - These determinations were carried out at weeks 13 (end of dosing) and 19 (end of recovery), using pooled samples of plasma, renal fat, kidneys, liver and brain of 5 rats/sex/group. The procedures used were detailed.

7. Necropsy and Histopathology - Complete necropsy and histopathology were performed on rats which died during the study and on those which were sacrificed at the termination of dosing and recovery periods. Tissues were fixed in formalin, embedded in wax, sectioned, and stained with hematoxylin and eosin. Tissue sections of kidneys, liver and heart were also stained with oil red 0 in order to demonstrate, apparently, the presence of fat. The following tissues were examined, using 1-3 sections/tissue: brain, eyes, salivary gland, thymus, lung, trachea, bone (with marrow), mesenteric lymph node, esophagus, small intestine, adrenal glands, liver, urinary bladder, testes, prostate, skin, skeletal muscle, aorta, spinal cord, pituitary gland, heart, thyroid gland, parathyroid glands, spleen, cervical lymph node, sciatic nerve, stomach, large intestine, pancreas, kidneys, ovaries, epididymides, uterus, mammary gland, and tongue.

8. Organ Weight (absolute and relative) - The following organs were weighed for all rats: adrenal and thyroid glands, ovaries, heart, liver, brain, testes and kidneys. Relative weights were expressed as organ/body weight ratio x 100 and as organ/brain weight x 100.

9. Statistical Analyses - All experimental results were analyzed statistically as is described below.

"- Univariate one-way analysis of variance was used whenever the variables could be assumed to follow a normal distribution. Pairwise t-tests based on a pooled variance estimate (single treatment groups against control.) were also applied.

- In case of obviously monotone (increasing or decreasing) dose-dependent relationship, the William's test (Biometrics, 27, 1971) was used to determine the lowest dose significantly different from the control group.

- In the absence of monotone dose-response relation and significant t-tests but with suggestive evidence of a difference between treatment and control, the contrast (overall mean of dose levels) to the control group was tested for significance (so called significance as a whole).

- The median test for overall or paired difference was used whenever the variables are defined on a discrete scale.

- Remarks:

  1) The adjustment for multiple testing is performed by comparing the expected and observed number of significant results obtained.

  2) The printing format of the summary tables requires rounding off procedures for mean, standard deviation and t-statistics. T-tests were calculated on the basis of exact values for means and pooled variances and then rounded off to two decimal places. Therefore, two groups may display the same printed means for a given parameter, yet display different t-statistics values with respect to teh control group.

  3) Where not specified explicitely, the significance level is 5%."

RESULTS

1. Toxic Signs and Mortality - Only one male rat from Group 4 died during the 4th week of the recovery period. Toxic signs were not observed in any group.

2. Body Weight - These data were reported separately for the male and female rats in three ways:

   a) Graphically (computer-constructed plots of mean body weights vs study time for each dose level).

   b) As summaries of the weekly mean values for the entire study, for each dose group.

   c) As individual body weights, also on the weekly basis, for the entire study (acclimation, dosing and recovery periods).

The summaries included also standard deviations, standard errors, minimal and maximal body weights, and number of animals weighed each week. The individual data included also numbers assigned to animals, mean body weights and standard deviations.

Male and female rats fed 0.2-20 ppm of lindane gained as much weight as the controls during the 12-week dosing time. Male and female rats fed 100 ppm of lindane (highest dose tested) gained, respectively, 8.4% and 14.9% less weight than the controls during the dosing time.

Lindane, at all levels fed, had no effect on the body weight gain of the female rats during the 6-week recovery period. However, the male rats fed 0.2-20 ppm and 100 ppm of lindane gained, respectively, 8.9-12.5% and 28.6% less weight during the recovery period, when compared with the controls. Data showing the weight profiles of the male and female rats used in this study are summarized in Tables 1 and 2.

Table 1. Mean Weight Gain (g) of Male Rats*

Group	1	2	3	4	5	6
Lindane (ppm)	0	0.2	0.8	4	20	100
Treatment						
Acclimation (1 wk.)	40	42	46	44	44	41
Dosing (12 wks.)	131	124	127	126	128	120
Percent decrease	—	5.4	3.1	2.3	2.3	8.4
Recovery (6 wks.)	56	51	50	49	50	40
Percent decrease	—	8.9	10.7	12.5	16.7	28.6

Table 2. Mean Weight Gain (g) of Female Rats*

Group	1	2	3	4	5	6
Lindane (ppm)	0	0.2	0.8	4	20	100
Treatment						
Acclimation (1 wk.)	13	15	13	12	12	9
Dosing (12 wks.)	47	54	52	51	45	40
Percent decrease	—	0	0	0	4.3	14.9
Recovery (6 wks.)	20	22	23	25	21	31
Percent decrease	—	0	0	0	0	0

*Weight gains were calculated by the reviewer, using the following values from the submission (Part 1, pp. 56-63; Accession No. 250340):

Acclimation period: Mean weight on day 4 (dosing week 1) minus mean weight on day 3 (acclimation week). Days 3 and 4 represent the first recordings of weight.

Dosing period: Mean weight for dosing week 12 minus mean weight for dosing week 1.

Recovery period: Mean weight for dosing-free week 6 minus mean weight for dosing-free week 1.

There were 20 rats of each sex/group during the acclimation and dosing periods. During the recovery period, each group had 5 female and 5 or 4 male rats. (One male rat from Group 4 died during the recovery week 4).

3. Food Consumption - These data were reported separately for male and female rats in three ways:

a) Graphically (computer-constructed plots of mean food intake vs study time for each dose level).

b) As summaries of the weekly mean food intake for each dose level, for the entire study.

c) As individual data, that is, as food intake per cage. (Rats were housed in groups of 5/cage in this study).

In each case, the food intake was reported as g/animal/day and as g/kg of body weight/day. The summaries included also standard deviations (not reported for the recovery period), standard error (not reported for the recovery period), maximal and minimal food intake and number of cages of animals used to weigh the food. The individual data included also numbers assigned to cages, treatment week and day, mean food consumption and standard deviations (not reported for the recovery period because only one cage of rats/sex/test group was used during that period).

Lindane, at all levels fed, had no effect on the food consumption of male and female rats during the dosing and the recovery periods, when food consumption was expressed either on the g/animal/day basis or on the g/kg of body weight basis. Decreases in the food intake with time in the lindane-treated groups and the controls, when food intake was expressed as g/kg of body weight/day (Table 4), reflected, probably, a gradually diminishing rate of growth of the animals. Data showing the food consumption of rats during the entire study are summarized in Tables 3 and 4.

Table 3. Mean Food Consumption of Male and Female Rats (g/animal/day)*

Lindane (ppm)	0	0.2	0.8	4	20	100
	MALES					
Treatment						
Acclimation (1 wk.)	21	21	22	21	22	21
Dosing (12 wks.)						
First 6 weeks	23	23	24	23	23	23
Second 6 weeks	23	22	24	24	24	24
Recovery (6 wks.)	22	21	24	22	23	22
	FEMALES					
Treatment						
Acclimation (1 wk.)	14	15	14	15	16	15
Dosing (12 wks.)						
First 6 weeks	17	17	17	17	18	16
Second 6 weeks	16	16	16	18	17	16
Recovery (6 wks.)	16	16	15	16	16	16

*Data in this table were calculated by the reviewer, using data reported in Part 1 (pp. 64-71) of the submission (Accession No. 250240).

Table 4. Mean Food Consumption of Male and Female Rats (g/kg of body weight/day)*

Lindane (ppm)	0	0.2	0.8	4	20	100
	MALES					
Treatment						
Acclimation (1 wk.)	92	96	96	95	98	93
Dosing (12 wks.)						
First 6 weeks	75	77	76	76	78	75
Second 6 weeks	61	62	62	63	65	64
Recovery (6 wks.)	55	56	57	56	56	58
	FEMALES					
Treatment						
Acclimation (1 wk.)	82	82	79	82	87	81
Dosing (12 wks.)						
First 6 weeks	82	80	79	84	84	80
Second 6 weeks	72	67	68	75	73	70
Recovery (6 wks.)	70	62	61	67	68	66

*Data in this table were calculated by the reviewer, using data reported in Part 1 (pp. 72-79) of the submission (Accession No. 250240).

4. Daily Ingestion of Lindane - Based on the diet analyses during the dosing weeks 1, 2, 6, 7, 8, 9 and 10, and the corresponding food intake and body weight data, the mean daily intake of lindane, calculated by the testing laboratory, was as follows:

Nominal levels of lindane in the diets of male and female rats (ppm)	Mean daily intake of lindane (mg/kg of body weight)	
	Male Rats	Female Rats
0	0	0
0.2	0.02	0.02
0.8	0.06	0.06
4	0.29	0.33
20	1.55	1.67
100	7.25	7.90

These data show that the female rats from Groups 4, 5 and 6 consumed slightly more lindane than did the males in the same groups.

The diets were generally homogenous with respect to lindane and lindane was stable in the diets under the conditions of this study. The greatest variations in lindane content, from one determination to another, were reported for Group 2 and 3. In the case of Group 2, the deviations from the nominal value (0.2 ppm) ranged from +10 to +60%. In the case of Group 3, the deviations from the nominal value (0.8 ppm) ranged from -50 to +60%.

5. Hematology - These data were reported separately for male and female rats in two ways:

a) As summaries of the mean values for the study weeks 1 (acclimation week), 6 and 13 (dosing weeks), and 19 (recovery week 6).

b) As individual values for the same time intervals.

In the case of the summaries, standard deviations, t-test significance, minimal and maximal values obtained for each parameter tested, and number of animals used for each test were also indicated. In the case of the individual values, ID numbers assigned to the animals, mean values and standard deviations were also reported.

Lindane, at all levels tested, had no effects on hematology of the male and female rats, when the lindane-treated animals were compared with the controls. Although there were dose-related decreases in the concentration of white blood cells (7.5-27%) and in the reticulocyte count (22-33%) of the male rats after 5 weeks of dosing with lindane, they did not occur after 12 weeks of dosing and during the recovery period. It is, therefore, difficult to say whether or not (or to what extent) these decreases could be attributed to lindane. In the case of the female rats, there were dose-unrelated increases in the lymphocyte count (11-21%) of Groups 4-6 at the conclusion of the recovery period, but not at other test intervals. It is, therefore, unlikely that these increases were due to lindane.

6. Clinical Biochemistry - These data were reported separately for male and female rats as individual values and as summaries of the mean values mostly for the study weeks 1, 6, 13 and 19. Carboxylesterase (aliesterase) and aminopyrine N-demethylase activities in the whole liver homogenates, and cytochrome P-450 content of the whole liver homogenates, were determined only during the study week 13 (end of dosing) and 19 (end of recovery). Brain tissue acetylcholinesterase activity was determined only at

the completion of dosing. The following enzyme activities were also reported graphically: liver aminopyrine N-demethylase, plasma and liver carboxylesterases, brain and erythrocyte acetylcholinesterases, and plasma butyrylcholinesterase. The cytochrome P-450 content of liver homogenates was also reported graphically. In the case of the summaries, standard deviations, t-test significance, minimal and maximal values obtained for each parameter tested, and number of animals used for each test were also indicated. In the case of the individual values, ID numbers assigned to the animals, mean values and standard deviations were also reported.

Except for the observations listed below, there was no difference in the parameters tested between the controls and the lindane-treated rats, both males and females. The following differences between the controls and the lindane-treated were observed:

a. Increases in the liver cytochrome P-450 levels at the termination of dosing, especially in the females. In the case of the females, the livers of the rats fed 0.8, 4, 20 and 100 ppm of lindane contained, respectively, 26, 42, 35 and 73% more of cytochrome P-450 than did the controls. All of these increases were considered statistically significant at the 5% or 1% level. In the case of the males, a 17% increase in the liver cytochrome P-450 content was observed only in the rats dosed with 100 ppm of lindane and was considered statistically insignificant. All of the increased cytochrome P-450 levels returned to the control values during the recovery period of six weeks.

   The testing laboratory regarded the increased cytochrome P-450 levels as being indicative of an enhanced induction of metabolizing (microsomal) enzymes. This could readily be expected in the lindane-fed rats, but the induction, if any, was very small in the male rats.

b. Plasma butyrylcholinesterase activity was increased in the male rats fed 20 and 100 ppm of lindane, and remained increased during the recovery period. The dose-related increases during the study weeks 6 (dosing), 13 (dosing) and 19 (recovery) were 26 and 37%, 15 and 27%, and 17 and 30%, respectively. Most of these increases were statistically significant at the 5% or 1% level, but the biological significance was not immediately apparent.

Plasma butyrylcholinesterase activity was not increased in the male rats from Groups 5 and 6, during the acclimation period. In the female rats, the plasma butyrylcholinesterase activity was 3-40% higher, in the dose-unrelated manner, than that of the controls during the lindane-free and the dosing periods.

c. There was a dose-related increase in the liver carboxylesterase activity of the female rats at the termination of the dosing, but not during the recovery period. Specifically, the increases were 8, 14, 28, 17 and 26% in the groups fed 0.2, 0.8, 4, 20 and 100 ppm of lindane, respectively. All of these increases but one (8%) were statistically significant at the 5 or 1% levels and reflected, probably, increased metabolism. The activity of this enzyme was not determined during the acclimation week and during dosing.

7. Urinalysis - These data were reported separately for the male and female rats as summaries of the mean values for the study weeks 1, 6, 13 and 19, and as individual values for the same time intervals.

Lindane, at all levels tested, had no effect on urinalysis.

8. Organ Weights - These data were reported as absolute weights and as relative weights (organ weight x100/body weight and organ weight x100/brain weight). Brain, heart, liver, kidneys, testes, ovaries, and adrenal and thyroid glands were weighed at the termination of dosing (15 rats/sex/group were sacrificed at that time) and at the termination of the recovery period, when the remaining 5 rats/sex/group were sacrificed.

There were small dose-related increases in the absolute weights of liver and kidneys (8-13%), in the liver and/or kidney to body weight ratios (7-14%), and in the liver and/or kidney to brain weight ratios (7-15%), all in the male and female rats fed 20 or 100 ppm of lindane. These rats were sacrificed at the termination of dosing.

After the recovery period, the absolute weights of liver and kidneys of the male and female rats did not generally differ from those of the controls. The above-mentioned liver and/or kidney to body weight (or brain weight) ratios were decreased from 5% to 17%, when compared with the controls. The decreases were dose-unrelated.

The weights or organs other than liver and kidneys were unaffected in this study.

9. Gross Necropsy Findings - These data were reported for individual animals, but were not summarized. Tables 5 and 6, prepared by the reviewer, contain all of the macroscopic findings that were reported.

Table 5. Macroscopic Findings Observed in Rats Sacrificed After 12-Weeks of Dosing with Lindane

Lindane ppm	Observation	Number of Rats Affected	
		M	F
0	Lung: Several dark red spots on all lobes.	1	
	Uterus filled with clear watery fluid.		3
0.2	Lung: Dark red foci on right lobe.	2	
	Opaque right cornea.	1	
	Small testes and reduced consistency.	1	
	Nodule in mesenteric adipose tissue.		1
	Kidney: Right dilated pelvis.		1
	Uterus filled with clear watery fluid.		1
0.8	Lung: Dark red foci on apical and/or median lobes.	2	
	Uterus filled with clear watery fluid.		3
4	Right kidney: White, gray and dark red in color.	1	
	Uterus filled with clear watery fluid.		3
20	Kidneys: Both covered with diffuse gray foci.	15	
	Liver: Hypertrophy	2	
	Lung: Dark red foci	1	1
	Uterus filled with clear watery fluid.		5
100	Kidneys: Both covered with diffuse gray foci.	15	
	Lungs: Dark red foci.	1	
	Lung (right phrenic lobe): Sharply demarcated red retraction.		2
	Uterus filled with clear watery fluid.		1

M = Males F = Females

These data show that lindane, at the 20 and 100 ppm levels, affected kidneys in all of the male rats. (Fifteen rats/sex/level were sacrificed after dosing).

Table 6. Macroscopic Findings Observed in Rats Sacrificed After 6-Weeks of the Recovery Period

Lindane ppm	Observation	Number of Rats Affected	
		M	F
0	Thymus: Right side dark red.	2	
0.2	Opacity in both eyes.		1
4	Dark red fluid in abdominal cavity.	1	
100	Kidney: Cyst in candal pole.	1	

M = Males F = Females

These data show that there were very few abnormalities in the rats (5/sex/dose) which received a lindane-free (control) diet, following a dietary exposure to different levels of lindane. It should be noted that kidneys of the male rats were no longer covered with diffuse gray foci, an abnormality observed in all of the male rats which were fed 20 or 100 ppm of lindane and were sacrificed immediately after dosing.

10. Histopathology - These data were reported in three ways:

a) As a summary, showing the incidence of microscopic findings in each test group.

b) As a tabulation of the individual microscopic findings in each tested group.

c) As the individual data, where the observations were described in detail for each animal. These data included also the exact duration of the exposure to lindane (for example, 84 or 88 days), the date of sacrifice (day, month, year) and the severity of a lesion (minimal, slight, moderate, marked or severe).

The most prevalent microscopic findings are summarized in Tables 7, 8, 9 and 10.

Table 7. Microscopic Findings Observed in Male Rats Sacrificed After 12-Weeks of Dosing with Lindane

Test Group	1	2	3	4	5	6
Lindane (ppm)	0	0.2	0.8	4	20	100
Observation	Number of Rat Affected					
Liver						
Round cell infiltration	6	6	4	7	5	6
Fatty degeneration (steatosis)	8	4	7	6	2	5
Kupffer cell proliferation			1	4	1	4
Hypertrophy				2	8	12
Glycogen storage	10	4	1	10	11	6
Kidneys						
Tubular degeneration					5	6
Hyaline droplets	10	11	14	15	15	15
Tubular casts	2	1	3	2	3	5
Tubular distension				1	11	13
Nephritis, interstitial			2		11	15
Basophilic tubules				1	14	15
Lungs						
Alveolar hemorrhage	5	6	4	2	1	1
Emphysema, (subpleural)	2	1	1	2	3	3
Spleen						
Hematopoiesis	3	5	10	6	1	1
Eyes						
Retroorbital hemorrhage	5	2	2	3	3	3
Retroorbititis	1	1	1	2	1	3
Harderian gland						
Dacreoadenitis	8	7	7	6	5	5
Trachea						
Cystic glands	3	4	4		5	3
Tracheitis	2	1	2	3	1	2
Parathyroid gland						
Interstitial fibrosis*	1/8	3/5	2/8	2/8	3/7	1/9

*In all instances, tissues from 15 animals were examined. In the case of the parathyroid gland, tissues from 5, 7, 8 or 9 animals only were examined.

According to these data, treatment-related changes were observed in the liver and kidneys. Hepatocellular hypertrophy in the male Groups 4, 5 and 6, and most of the renal changes occurring in the Groups 2, 3 and 4 were minimal or slight. As the levels of lindane were increased to 20 and 100 ppm, the renal changes increased in severity. This was very evident especially in the case of hyaline droplets in the proximal tubular epithelium. In the Groups 2, 3, 4, 5 and 6, hyaline droplets were mostly minimal, slight, moderate, marked and severe, respectively.

Table 8. Microscopic Findings Observed in Male Rats Sacrificed After a 6-Month Recovery Period*

Test Group	1	2	3	4	5	6
Lindane (ppm)	0	0.2	0.8	4	20	100
Observation	Number of Rat Affected					
Liver						
Round cell infiltration	2	2	1	2		
Fatty degeneration (steatosis)	2	2	2			2
Glycogen storage	5	5	5	4	5	5
Kidneys						
Hyaline droplets	2	4	4	5	3	5
Tubular casts	1		1	1	3	1
Tubular distension					2	4
Nephritis, interstitial					4	5
Basophilic tubules				1	3	5
Nephroblastoma				1		
Lungs						
Alveolar hemorrhage	1					
Emphysema	1	1				
Spleen						
Hematopoiesis	3	2	2	3		

Table 8 continued on next page.

Table 8, continued

Test Group	1	2	3	4	5	6
Lindane (ppm)	0	0.2	0.8	4	20	100
Observation	Number of Rat Affected					
Eyes						
Retroorbital hemorrhage	1		1	1	1	2
Retroorbititis	2	1		1	1	3
Harderian gland						
Dacreoadenitis	2	3	2	3	3	1
Trachea						
Cystic glands	2	2		1	1	2
Lymph node						
Hyperplasia			1			
Thymus						
Cysts		1			3	1

*In all instances but one (Group 4), tissues from 5 animals/dose were examined. In the case of Group 4, one male rat died during the recovery week 4.

These data show that lindane, especially at the 20 and 100 ppm levels, caused an irriversible or a long-lasting damage to the kidneys of male rats. Although tubular degeneration was absent at the termination of a 6-week recovery period, all of the toxic symptoms noted at the completion of dosing were sill present and with approximately the same frequency. However, these symptoms were only slight to moderate in severity. The absence of hepatocellula hypertrophy at the termination of the recovery period suggested the following: a) that this toxicity was reversible; and b) that hepatocellular hypertrophy was not a toxic effect, but was indicative of an induction of metabolizing (microsomal) enzymes, as was presumed by the testing laboratory.

Table 9. Microscopic Findings Observed in Female Rats Sacrificed After 12-Weeks of Dosing with Lindane

Test Group	1	2	3	4	5	6
Lindane (ppm)	0	0.2	0.8	4	20	100
Observation	Number of Rat Affected					
Liver						
Round cell infiltration	4	7	5	6	6	5
Fatty degeneration (steatosis)	2	3	2	2	6	1
Kupffer cell proliferation		3	1	1	1	4
Hypertrophy					6	9
Glycogen storage		2	1	4	3	
Kidneys						
Tubular degeneration		1			5	5
Hyaline droplets		2		4	2	4
Tubular casts	1	1			3	1
Nephritis, interstitial		1			1	1
Mineralization	7	4	4	5	6	7
Lungs						
Alveolar hemorrhage	1	1		1	3	4
Emphysema, subpleural	2	1	3	2	1	4
Spleen						
Hematopoiesis	8	12	11	10	10	10
Eyes						
Retroorbital hemorrhage	3	3			2	2
Retroorbititis		1	1		1	5
Harderian gland						
Dacreoadenitis	9	7	7	7	9	9

Table 9 continued on next page.

Table 9, continued

Test Group	1	2	3	4	5	6
Lindane (ppm)	0	0.2	0.8	4	20	100
Observation	Number of Rat Affected					
Trachea						
Cystic glands	7	5	4	6	6	3
Tracheitis				1	1	4
Parathyroid gland						
Interstitial fibrosis*	1/8	2/6	2/12	3/8		1/13
Thymus						
Cysts	1	2	3	4	3	3
Pituitary gland						
Cysts		1	2	2		
Ovaries						
Cysts			2	1	1	1
Uterus						
Distension	3	2	4	4	6	3

*In all instances, tissues from 15 animals/dose were examined. In the case of the parathyroid gland, tissues from 6, 8, 12 or 13 animals/dose were examined.

According to these data, lindane, at the 20 and 100 ppm levels, caused hepatocellular hypertrophy in 40 and 60% of the female rats, respectively. The same levels of lindane caused also renal tubular degeneration in about 33% of the animals which were sacrificed immediately after the dosing. The renal changes were, therefore, much less severe than those reported for the male rats. At the 100 ppm level of lindane, toxic effects were also observed in the eyes (hemorrhage and retroorbititis), trachea (tracheitis) and lungs (alveolar hemorrhage and emphysema) of the female rats. Changes in the eys, trachea and lungs were seen in about 27-33% of the animals.

Table 10. Microscopic Findings Observed in Female Rats Sacrificed After a 6-Week Recovery Period*

Test Group	1	2	3	4	5	6
Lindane (ppm)	0	0.2	0.8	4	20	100
Observation	Number of Rat Affected					
Liver						
Round cell infiltration	4	2	2			2
Fatty degeneration (steatosis)	1	2	1		5	2
Glycogen storage	5	4	5	5	4	5
Kidneys						
Hyaline droplets	1	1	1	2	1	1
Nephritis, interstitial				1		1
Mineralization	2	4	3	4	3	2
Lungs						
Emphysema	1			1		2
Spleen						
Hematopoiesis	3	5	5	5	5	5

Table 10, continued on next page.

Table 10 continued

Test Group	1	2	3	4	5	6
Lindane (ppm)	0	0.2	0.8	4	20	100
Observation	Number of Rat Affected					
Eyes						
Retroorbital hemorrhage				1		
Retroorbititis			1	1		
Harderian gland						
Dacreoadenitis	4	3	4	4	2	4
Trachea						
Cystic glands	2	1	2	4	2	2
Thymus						
Cysts	3	3	3	1	4	3
Lymph node						
Hyperplasia				1		

*In all instances, tissues from 5 animals/dose were examined.

These data show that the histopathological findings observed in the lindane-treated rats at the termination of the recovery period did not differ from those observed in the controls. Considering the histopathology findings obtained for the female rats at the termination of dosing (Table 9), this means that hepatocellular hypertrophy was probably not a permanent effect, but reflected indeed the induction of microsomal enzymes.

The presence of renal tubular degeneration in the male and female rats (Groups 5 and 6) at the termination of dosing with lindane (Tables 7 and 9) and its absence after the recovery period (Tables 8 and 10) suggest that tubular degeneration was probably a reversible toxic effect.

11. Levels of Lindane in Plasma

These determinations were performed by gas-liquid chromatography (GLC) on 5 rats from each sex and group at the termination of dosing and after the recovery period.

There was a dose-related increase in the plasma levels of lindane at the termination of dosing, in both males and females. After the recovery period, the male rats from Groups 2-6 and the female rats from Groups 2-4 had as much lindane in plasma as did the controls (Group 1). The lindane content of plasma of the female rats from Groups 5 and 6 was slightly higher than that of the controls. These data are summarized in Table 11.

Table 11. Levels of Lindane in Plasma

Test Group	1	2	3	4	5	6
Lindane (ppm)	0	0.2	0.8	4	20	100
Test Interval	ng of Lindane/ml of Plasma					
After dosing - Males	<1	13.9	61.8	55.0	646.7*	257*
- Females	<1	5.5	18.4	127.8	121.7	408
After recovery - Males	<1	<1	<1	<1	<1	<1
- Females	<1	<1	<1	<1	1.3	1.5

*It appears as though these values were reversed during the tabulation of the data.

The concentration of lindane in plasma was calculated from the standard curve, obtained with 0, 5, 50 and 100 ng of lindane/ml (also of plasma, rather than water). Since trace amounts of lindane were detected in plasma without added lindane, this means that the Kliba 343 Rats/Mouse Maintenance Diet was probably the source of

that lindane. The procedure used to detect lindane in feed had a sensitivity limit of 5 ppb (or 5 mg of lindane/g of feed) and none was detected. According to this submission (Part 1, p. 33), a maximal EPA-specified level of lindane in (rodent?) feed is 20 ppb and "results exceeding EPA levels up to 25% are not regarded as of toxicological relevance". Although it was not specified, the plasma used in the construction of the standard curve was apparently that obtained from the control rats. Drinking water was not analyzed for lindane.

12. Levels of Lindane in Tissue

These determinations were performed on pooled samples of tissues of equal weights, obtained from each sex and group at the completion of the dosing and the recovery period. Prior to analysis by GLC, tissue samples (liver, kidneys, renal dat and brain) were frozen in liquid nitrogen and stored at -20°C. Data concerning the levels of lindane in tissues are summarized in Tables 12 and 13.

Table 12. Levels of Lindane in Tissues of Male Rats

Test Group	1	2	3	4	5	6
Lindane (ppm)	0	0.2	0.8	4	20	100
Tissue		After Dosing; ppm				
Liver	0.003	0.052	0.113	0.182	2.111	1.355
Kidney	0.012	0.052	0.908	9.832	7.859	83.749
Renal fat	0.001	0.199	0.139	0.408	2.579	11.394
Brain	0.003	0.083	0.109	0.410	2.338	3.841
	After Recovery; ppm					
Liver	0.010	0.001	0	0.004	0.049	0.008
Kidney	0.025	0.010	0.006	0.011	0.023	0.037
Renal fat	0.010	0.034	0.003	0.005	0.008	0.024
Brain	0.010	0.002	0.002	0.005	0.006	0.020

These data show that the concentrations of lindane in the tissues of male rats were dose-dependent. The highest levels of lindane were detected in the kidneys. During a 6-week recovery period, when the animals received lindane-free diets, the tissue levels of lindane in all test groups (Groups 1-6) were about the same. Small amounts of lindane, found in the tissues of the control rats, orignated, probably, from the Kliba 343 Rat/Mouse Maintenance Diet.

Table 13. Levels of Lindane in Tissues of Female Rats

Test Group	1	2	3	4	5	6
Lindane (ppm)	0	0.2	0.8	4	20	100
Tissue		After Dosing; ppm				
Liver	0.008	0.028	0.035	0.346	1.957	3.922
Kidney	0.092	0.012	0.034	0.254	2.077	4.398
Renal fat	0.008	0.190	0.481	1.760	15.220	28.136
Brain	0.002	0.021	0.030	0.198	1.472	2.895
	After Recovery; ppm					
Liver	0.008	0.001	0.001	0.005	0.009	0.028
Kidney	0.008	0.002	0	0.003	0.010	0.001
Renal fat	0.047	0.008	0.006	0.011	0.056	0.094
Brain	0.004	0.001	0.001	0.005	0.006	0.017

These data show that the concentration of lindane in the tissues of female rats were dose-dependent. The highest levels of lindane occurred in the renal fat. During the recovery period, the tissue levels of lindane in all test groups (Groups 1-6) were similar.

CONCLUSIONS:

Wistar KFM-Han (outbred) SPF rats, 20 males and 20 females/level, were fed diets containing the following levels of lindane: 0, 0.2, 0.8, 4, 20 and 100 ppm. The feeding was started after one week of an acclimation period and was continued for 12 weeks, at which time 15 rats from each group were sacrificed. The remaining 5 male and 5 female rats from the lindane-fed groups were placed on a lindane-free (control) diet for 6 weeks (recovery period) and then were sacrificed. The remaining 5 male and 5 female rats from the control groups were also continued on their diet for the additional 6 weeks before they were sacrificed.

The following parameters were studied during the acclimation, dosing and recovery periods: observations for toxic signs and mortality, body weights, food consumption, hematology, clinical biochemistry, urinalysis, organ weights, organ to body weight and organ to brain weight ratios, gross necropsy, histopathology and levels of lindane in plasma, liver, kidneys, renal fat and brain.

The following results were obtained:

1. Lindane, at all levels tested, had no effect on the mortality, food consumption, hematology and urinalysis, and no toxic signs were observed in any group.

2. Male and female rats fed 100 ppm of lindane (highest level tested) gained, respectively, 8.4 and 14.9% less weight during the dosing period than did the controls.

3. Male rats fed 0.2-20 ppm and 100 ppm of lindane gained, respectively, 8.9-12.5% and 28.6% less weight during the recovery period, when compared with the controls. Lindane, at all levels tested, had no effect on the body weight gain of the female rats during the recovery period.

4. There were increases in the liver cytochrome P-450 levels at the termination of dosing, especially in the female rats. Females fed 0.8, 4, 20 and 100 ppm of lindane, had, respectively, 26, 42, 35 and 73% more of the hepatic cytochrome P-450 than did the controls. In the case of the males, a 17% increase in the liver cytochrome P-450 content was observed only in the rats dosed with 100 ppm of lindane. All of the increased hepatic cytochrome P-450 levels returned to the control values during the recovery period. The augmentation in the hepatic cytochrome P-450 content during dosing is regarded as an induction of the microsomal detoxyfying enzymes, and not as a toxic symptom.

5. There was a dose-unrelated increase in the liver carboxylase activity of the female rats at the termination of dosing, but not at the termination of the recovery period. Specifically, the increases were 8, 14, 28, 17 and 26% in the groups fed 0.2, 0.8, 4, 20 and 100 ppm of lindane, respectively. The enzyme was unaffected by lindane in the male rats.

6. There were small dose-related increases in the absolute weights of liver and kidneys (8-13%), in the liver and/or kidney to body weight ratios (7-14%), and in the liver and/or kidney to brain weight ratios (7-15%), all in the male and female rats fed 20 or 100 pm of lindane.

7. Gross necropsy had revealed that lindane, at the 20 and 100 ppm levels, affected both kidneys of all male rats (or 15 rats/group), examined at the termination of dosing. These kidneys were covered with diffuse gray foci. However, this abnormality was reversible because the kidneys of all male rats (or 5 rats/group) which were sacrificed at the termination of the recovery period did not have foci. Gross necrospy revealed nothing remarkable in the female rats at the termination of the dosing and the recovery period.

8. Histopathology had revealed that lindane caused changes in the kidneys and liver of the male and female rats. These changes occurred chiefly in the groups fed 20 or 100 ppm of lindane. The changes were frequent, dose-related and severe in the males, and infrequent, dose-unrelated and generally mild in the females. The following renal changes were observed: tubular degeneration, hyaline droplets, tubular casts, tubular distension, interstitial nephritis and basophilic tubules. Hypertrophy was the only dose-related liver change in both males and females.

Most of the renal changes, but not tubular degeneration, were observed also at the termination of the recovery period. Tubular degeneration was not observed at that time and neither was liver hypertrophy, meaning that liver hypertrophy was, apparently, a consequence of enzyme induction.

9. There was a dose-related increase in the plasma level of lindane, in the male and female rats, at the termination of dosing, but there was no lindane in plasma (beyond the control levels) at the termination of the recovery period. Similar findings were observed for liver, kidney, renal fat and brain. The highest levels of lindane were found in the kidneys of the male rats and in the renal fat of the female rats.

10. NOEL: 4 ppm, for both male and female rats. This dosage level is equivalent to 0.3 mg/kg body weight/day - based on diet analyses, food intake and body weight data (see page 9 of this review).

Although some renal changes occurred at this level, they were generally mild and were generally single occurences. Other changes observed at the 4 ppm level, such as liver hypertrophy and increased levels of hepatic cytochrome P-450, were not toxic manifestations.

LEL: 20 ppm (next highest level tested), for both male and female rats. Permanent kidney damage was observed at this level.

CORE CLASSIFICATION: Guideline

COMMENT:

This study was conducted in Switzerland and this submission is a translation from a foreign language. It is a well planned, well conducted and very well reported study. It was a pleasure, indeed, to evaluate such a beautiful study. Not a single detail was left out and the individual data agreed with the summaries.

There is one point, however, which may cause confusion to those who just want "to take a look" here and there in the report, without becoming familiar with the entire report.

As was already indicated, this study consisted of the acclimation period (1 week), dosing period (12 weeks) and the recovery period (6 weeks), or a total of 19 weeks. Unfortunately, these simple test intervals are sometimes lost in the report when 1) the acclimation week is regarded as the first treatment week or 2) when the acclimation week is forgotten. We thus have a study lasting 18 or 19 weeks, dosing period of 12- or 13 weeks duration and, in one instance, a recovery period lasting 5 weeks. Three illustrations appear below:

1. "Total duration of dosing: 12 weeks." (Part 1, p. 9).

2. "------- the concentration of cytochrome P-450 after 13 weeks of treatment". (Part 1, p. 10).

3. "This report describes ------ the concentration of Lindane in tissues ------ obtained from rats exposed to 13 weeks of continuous uptake of Lindane in feed and from rats of a 5 weeks depletion period -------". (Part 2, p. 1125).

However, this reviewer regards these "discrepancies", resulting probably from the translation, as insignificant in the otherwise excellent report.

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TS-769:th:TOX/HED:KKLocke:6-14-83:card misc. #29

6/16/83

STUDY TYPE: Metabolism (Mouse)

STUDY TITLE: Covalent binding to mouse liver DNA as inconclusive mechanism of carcinogenic action of hexachlorocyclohexane isomers. (No study number.)

ACCESSION NO.: 250339 TOX. CHEM. NO.: 527

RECORD NO.: 98077

SPONSOR: Centre International d'Etudes an Lindane (C.I.E.L.), Brussels, Belgium.

TESTING LABORATORY: Institute of Toxicology, ETH and University of Zürich, Switzerland.

DATE OF FINAL REPORT: April 15, 1983

TEST MATERIALS: Alpha, beta, gamma and delta isomers of ^{3}H-hexachlorocyclohexane (HCH) and ^{14}C-thymidine

PROTOCOL:

Groups of NMRI, CF1 and C6B3F1 young male mice (25-40 g) were acclimated for one week and then were given (by gavage) single doses of alpha, beta, gamma or delta ^{3}H-hexachlorocyclohexane, identified as alpha-(^{3}H)HCH, beta-(^{3}H)HCH, gamma-(^{3}H)HCH or lindane, and delta-(^{3}H)HCH, respectively. In order to determine time-dependent binding of (^{3}H)HCH to DNA, 2 mice from each group were sacrificed after 10 hours and after 1, 2, 5 and 10 days, following dosing with the four isomers of (^{3}H)HCH. In order to determine the rate of DNA synthesis, other male mice received an intraperitoneal injection of ^{14}C-thymidine 6.5 hours after the administration of (^{3}H)HCH, and were sacrificed 3.5 hours later. DNA and HCH metabolites were then isolated from the livers of the mice, and were checked for radioactivity. Deoxynucleosides were subsequently isolated from DNA. A great variety of procedures were used to determine the purity (chemical and radiopurity) of

the (^{3}H)HCH isomers which were synthesized in the testing laboratory. A great variety of procedures were also used to characterize various fractions obtained from the livers of the ^{3}H- and ^{14}C-treated mice.

RESULTS AND CONCLUSIONS:

This submission is a copy of a manuscript which was submitted for publication in "Carcinogenesis". The manuscript is authored by Peter Sagelsdorff, Werner K. Lutz and Christian Schlatter.

The objective of this study was to determine whether or not the tumor formation by HCH required covalent binding of HCH to DNA. This was accomplished by using strains of mice with different susceptibility to the oncogenic ("tumorigenic") action of HCH and by using HCH isomers with varying oncogenic potential. Binding was evaluated in terms of the Covalent Binding Index (CBI), that is, CBI = DNA damage/dose or (umoles of bound HCH/mole of DNA nucleotide)/(mmole of HCH administered/kg body weight). The following results were obtained:

1. All strains of mice had similar CBIs, although the NMRI mice were not susceptible to the tumor-inducing action of gamma-HCH (lindane).

2. Alpha, gamma and delta isomers of HCH all gave very low and similar CBIs, although the alpha isomer is a more potent carcinogen (induces liver tumors in rats) than the gamma isomer and the delta isomer never induced tumors. Hardly any binding of beta-HCH to DNA was observed.

3. There was a tendancy for the alpha isomer of HCH to induce a higher rate of DNA synthesis.

It was concluded that the covalent binding of HCH to DNA, or the damage of DNA by HCH, does not occur with any degree of significance in the livers of mice. Hexachlorocyclohexane isomers, including lindane, are therefore non-mutagenic or non-genotoxic liver carcinogens, according to the authors.

CLASSIFICATION OF STUDY: Acceptable. There are no Core criteria for this kind of a study.

Krystyna K. Locke

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6/16/83

TS-769:th:TOX/HED:KKLocke:6-15-83:card misc.#29

Tox Chem No. 527
~~257~~

Study	Material	EPA Accession No.	Results	NOEL / LEL / LD50 / LC50 / PIS	Tox Category	Core Grade
Subchronic oral (90 days), rat. Research and Consulting Company Ltd., Switzerland. Study No. 005220 February 3, 1983	Lindane 99.85% pure	[redacted] 250340 250341 250342	NOEL: 4 ppm (male and female rats), based on systemic effects. LEL: 20 ppm (male and female rats). Permanent or long-lasting kidney damage occurred at this level. Levels of lindane fed in diet: 0, 0.2, 0.8, 4, 20 or 100 ppm. Protocol: Wistar KFM-Han (outbred) SPF rats, 20 M + 20 F / level, were fed lindane for 12 weeks, at which time 15 M + 15 F rats from each group were sacrificed. The remaining 5 M + 5 F rats from each group were then fed a control diet for 6 weeks (recovery period) and were subsequently sacrificed. Parameters studied during the acclimation (1 week), dosing and/or recovery periods: observation for toxic signs, mortality, body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weights, organ/body wgt. and organ/brain wgt. ratios, gross necropsy, histopathology, and lindane levels in plasma, liver, kidneys, renal fat and brain. This is an excellent study.		—	1 Guideline

This dosage level is equivalent to 0.3 mg/kg/day based on diet analyses, food intake and body weight data.

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Ldd 1/83

Tox Chem No. 527

Study	Material	EPA Accession No.	Results	NOEL / LEL / LD50 / LC50 / PIS	Tox Category	CORE Grade
Metabolic, mouse. Institute of Toxicology, ETH and Univ. of Zürich, Switzerland (No study number) April 15, 1983	3H-hexachlorocyclohexane (α, β, γ and Δ isomers) and 14C-thymidine	250339	The objective of this study was to determine whether or not the tumor formation by hexachlorocyclohexane (HCH) required covalent binding to DNA. (Gamma isomer of HCH is lindane). It was concluded that α and γ isomers of HCH were non-mutagenic liver carcinogens, meaning that they did not bind to DNA (that is, they did not damage it). In this study, young male NMRI, CFI and C6B3F1 mice were ~~given~~ orally different 3H-labeled isomers of HCH with or without 14C-thymidine. Single doses of HCH were used. The mice, 2/treatment, were then sacrificed at different time intervals and DNA was isolated from the livers and studied by a great variety of procedures. According to this study, β and Δ isomers of HCH were neither mutagenic nor oncogenic.	—		1 Acceptable

R.H. 1/8