

US EPA ARCHIVE DOCUMENT

DATA EVALUATION REPORT

LINDANE
(gamma HCH)

STUDY TYPE: SUBCHRONIC ORAL NEUROTOXICITY – RAT (82-7)

MRID 44781101

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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LINDANE

Subchronic Oral Neurotoxicity (82-7)

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Reregistration Branch 4 (7509C)

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Reregistration Branch 4 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Neurotoxicity – Rat
OPPTS 870.6200 [§82-7]

DP BARCODE: D254526

P.C. CODE: 009001

MRID: 44781101

SUBMISSION CODE: S559140

CASE NO.: 818566

TEST MATERIAL: Lindane (gamma HCH)

SYNONYMS: 1,2,4,5/3,6-gamma stereo Isomer of 1,2,3,4,5,6,-hexachlorocyclohexane (58-89-9)

CITATION: Hughes, E.W., 1999. 13-week neurotoxicity study in rats by dietary administration. Huntingdon Life Sciences Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Project Identity CIL/012, March 5, 1999, MRID 44781101, Unpublished.

SPONSORS: C.I.E.L. (Centre International d'Etudes du Lindane), 56 rue des Colonies (Box 14), B-1000 Bruxelles, Belgium.

EXECUTIVE SUMMARY: In a subchronic oral neurotoxicity study (MRID 44781101), groups of 10 CrI:CD@BR rats/sex/group were administered Lindane (Batch No. HLS96/1, Purity 99.78%) in the diet for 13 weeks at concentrations of 0 (control), 20, 100, or 500 ppm. Due to severe toxic reactions to treatment at 500 ppm, the dose was reduced to 400 ppm on day 11 of treatment thereafter. These doses resulted in average daily intake values of 0, 1.4, 7.1, and 28.1 mg/kg/day for males and 0, 1.6, 7.9, and 30.2 mg/kg/day in females for 0, 20, 100, and 500/400 ppm, respectively. Functional observational battery (FOB) and motor activity (MA) tests were performed prior to administration and after 4, 8, and 13 weeks of treatment. Body weights were recorded pre-test, weekly during the study period and on FOB assessment days. Clinical signs were recorded at least once daily. At study termination all animals were sacrificed and fixed by whole body perfusion and designated tissues of the nervous system were processed for microscopic neuropathological evaluation.

Three females in the 500/400 group died prior to scheduled termination. These deaths were attributed to treatment with Lindane. One death was recorded on Day 11 of the study, one during week 10 and one during week 13. Clinical signs prior to death included weight loss, swollen muzzle with scabbing, hunched posture, piloerection, and staining of the anogenital region.

Observations in surviving females treated at 500/400 ppm were hypersensitivity to touch, staining of the urogenital region, and scabbing of the toes.

Significant treatment-related decreases ($p < 0.05$ or $p < 0.01$) in body weight were observed among males and females treated with 500/400 ppm of 14% and 23%, respectively. Decreases in body weight gains (70% ♂ and 180% ♀, $p < 0.01$), food consumption (35% ♂ and 50% ♀, $p < 0.05$ or $p < 0.01$, respectively), and food conversion ratios were observed for males and females in the 500 ppm groups compared to the control group for the first week of the study. Male rats tended to recover from these effects after the dose was lowered. Females, however, did not exhibit this same level of recovery as their food consumption remained slightly depressed throughout the remainder of the study.

Females in the 100 ppm group had significantly decreased body weight gains (40%, $p < 0.05$) compared to the control group during the first week of the study and this effect continued, although not at a level of significance throughout the remainder of the study. Females in the 100 ppm group had significantly decreased food consumption (16%, $p < 0.01$) for the first week of the study and this trend continued throughout the study. Liver weights were also found to be increased at 500/400 ppm for both sexes; no additional information was given.

During the FOB assessment (table A is attached at the end of this document), males and females treated at the highest dose (500/400 ppm) were perceived as difficult to handle. They also were observed to have piloerection and hunched posture. Females in the highest dose group had missing claws (3), tended to urinate more often than controls, had a higher incidence of grooming behavior, rearing, motor activity, and one female was observed to convulse. Females across the dose groups were observed walking on tiptoes (5-7) and these incidences were significantly increased compared to the control (1) for the highest dose group. Females (5) in the 100 ppm group also had increased incidences of grooming behavior at the Week 4 evaluation and one animal in this group was extremely difficult to handle.

The assessments of forelimb and hindlimb grip strength as well as hindlimb splay revealed no differences for any of the treated groups compared to the control groups. Colburn motor activity was also similar among treated groups compared to the control groups.

No neuropathological endpoints attributable to Lindane administration were observed during the histological examinations of the peripheral or central nervous systems of these animals at any exposure concentration.

The NOAEL for systemic toxicity is 100 ppm for males (7.1 mg/kg) and 20 ppm for females (1.6 mg/kg). Based on the substance-related effects on body weight, body weight gain, food consumption, and clinical signs of toxicity the LOAEL levels for systemic toxicity in males is 500/400 ppm (28.1 mg/kg) and 100 ppm for females (7.9 mg/kg).

The NOAEL for neurotoxic effects is 100 ppm for males (7.1 mg/kg) and females (7.9 mg/kg). The neurotoxicity LOAEL is 500/400 ppm based on hypersensitivity to touch and hunched posture.

This study is classified **Acceptable/guideline** and satisfies the Subdivision F guideline requirement for an acute oral neurotoxicity study (§81-8) in rats.

COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, and Good Laboratory Practice Compliance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: Lindane

Description: colorless, faint to odorless, crystalline solid

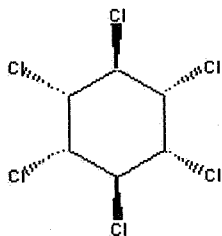
CAS No.: 58-89-9

Lot/Batch No.: Batch No. HLS96/1

Purity: 99.78%

Contaminants: none given

Structure:



2. Vehicle

None, administered in the diet.

3. Test animals

Species: rat

Strain: CrI: CD@BR

Age and mean weight at study initiation: 35 day old males within 15 g weight range and 35 day old females within 13 g weight range

Source: Charles River Breeding Laboratories, Manston Road, Margate, Kent, England

Housing: individually, in suspended wire mesh stainless steel cages

Food: SDS Rat and Mouse No. 1 maintenance diet. Available *ad libitum*

Water: Filtered tap water was available *ad libitum* from an automatic watering system.

Environmental conditions:

Temperature: 22 ± 3 °C

Humidity: $50 \pm 10\%$

Air changes: not given

Photoperiod: 12 hr light/12 hr dark

Acclimation period: 12 days

B. STUDY DESIGN

1. In life dates

Start: June 15, 1998

End: September 18, 1998

2. Animal assignment

All animals were received on June 3, 1998. The number of animals assigned to the exposure groups is listed in Table 1. Animals were randomly assigned to groups based on body weight, 10 rats/gender were selected for assignment to each of four treatment groups.

TABLE 1. Study Design			
Test group/color code	Dietary Conc. (ppm) Lindane	Dose (mg/kg) Lindane/Number of animals	
		Males	Females
1. White	0.00	0/ 10	0/ 10
2. Yellow	20	1.4/ 10	1.6/ 10
3. Blue	100	7.1/ 10	7.9/ 10
4. Pink	500/400*	28.1/ 10	30.2/ 10

Data taken from pp. 15 and 34, MRID 44781101.

*Treatment reduced from 500 to 400 ppm on Day 11.

3. Validation of test methods

None provided.

4. Rationale for dose selection

The doses selected for this study were selected by the sponsor after a review of existing publications including long term dietary studies which had utilized a top dosage of 400 ppm. Some clinical signs were observed at 400 ppm, but only after

lengthy exposures. After discussions between the Sponsor and the EPA, a maximum dosage of 500 ppm was selected for the current study.

5. Preparation and analysis of test diet

Lindane was administered to the animals by mixing the test material with powdered diet which was then fed to the animals. Once a week, the test substance was ground into the diet then blended for at least 2 minutes in a Turbula mixer to form a uniform premix. Subsequently, appropriate amounts of food were added to this premix in order to obtain the desired test concentrations and mixed for about 5 minutes in a Turbula mixer. Diets were prepared weekly. Samples of diets prepared in Weeks 1, 6, and 12 were analyzed for concentration of the test material. Homogeneity of the mixing process and chemical stability were assessed for an associated study (Schedule no. CIL/014) which was conducted by the same laboratory.

Results

Homogeneity analysis. Samples of diets containing Lindane taken from preparations in Study CIL/014 were within 8 % (C.V.) of the intended concentrations of 1000 ppm (mean 1000 ± 25.8) and 10 ppm (mean 10.1 ± 0.750).

Target concentration. The actual concentrations for samples taken from diet preparations were assessed for this study (MRID 44781101) and found to be within 8% of the nominal concentration. For Week 1, target concentrations of 0, 20, 100, and 500 ppm were found to have actual concentrations of 0, 19.5, 98.8, and 484 ppm, respectively. For Week 2, samples with a target concentration 400 ppm were found to have a mean actual concentration of 400 ppm. Mean actual concentrations for samples taken from Weeks 6 and 12 were 18.4, 100, and 393 ppm; and 18.7, 96.5, and 385 ppm for target concentrations of 20, 100, and 400 ppm, respectively.

Stability. The stability of Lindane stored at room temperature (21°C) for up to 22 days was assessed for Study CIL/014. For an intended concentration of 10 ppm, the actual concentration after 8 days was 9.41 ± 0.191 and after 22 days was 9.44 ± 0.049 with an apparent first order rate constant of 0.00328 ppm/day and an estimated shelf life of > 22 days. For an intended concentration of 1000 ppm, the concentration after 8 days at room temperature was 974 ± 30.4 and after 22 days was 945 ± 32.5 with an apparent first order rate constant of 0.00261 ppm/day and an estimated shelf-life of > 22 days.

Conclusion. These analyses confirm that the method of diet preparation for this study yields homogeneously mixed diets and that Lindane is stable in diet preparations at room temperature for > 22 days. The nominal concentrations were representative of actual dietary concentrations fed to the animals.

6. Statistical analysis

Statistical analyses were carried out separately for males and females.

The individual animal was considered as the basic experimental unit.

Food consumption data were analyzed based on weekly totals and body weight data were analyzed using weight gains. Bartlett's test was applied to determine heterogeneity of variance between treatments. A one-way analysis of variance was conducted followed by Student's t test and Williams' test.

The Functional Observation Battery (FOB), Motor Activity (MA), and body weight data were analyzed using a one-way analysis of variance model with Williams' test for a dose-related effect. When a significant difference between controls and treated groups was suggested, the Jonckheere-Terpstra test was used for confirmation.

All data were tested at the $p \leq 0.05$ or $p \leq 0.01$ level.

C. METHODS

1. Observations

Cage-side observations for gross signs of substance-related effects were conducted for animals in all groups once daily, and animals were further observed for mortality and moribundity twice daily throughout the study. Rats were individually handled and observed for abnormal behavior and appearance once pretest and during FOB assessments.

2. Body weight

Body weights were recorded once pretest, on the day of treatment commencement, and once a week thereafter. Body weights were also recorded on the days of FOB assessment.

3. Food consumption and food conversion ratios

Food consumption and food conversion ratios were determined weekly.

Food consumption (g/rat/week) = $\frac{\text{Total food given} - \text{Total food left}}{\text{# of animal days}^*}$

Food conversion ratio = Food consumed / Body weight gain

* 1 animal day for each animal alive for a whole day

4. Functional observational battery (FOB)

Rats were subjected to a FOB prior to exposure and during the 4th, 8th, and 13th week of treatment. Not all rats were tested on the same day, but testing was balanced across groups.

a. Home cage observations

Animals were observed in their closed home cages for posture, tremor, twitches, spontaneous vocalizations, convulsions, and palpebral closure.

b. Handling observations

Observations during handling from the cage to the open arena were made for convulsions, tremors, twitches, salivation/lacrimation, palpebral closure, exophthalmus, piloerection, vocalization, and ease of removal and handling.

c. Open arena observations

Animals were observed in an open-arena with the following parameters recorded: convulsions/tremors/twitching, bizarre or stereotypic behavior, grooming, number of rearings, gait, arousal, respiration, defecation, and urination.

d. Sensorimotor tests/reflexes

When the animals were removed from the open field, they were subjected to the following sensorimotor or reflex tests: approach response, touch response, startle response, righting reflex, tail pinch, pupil response, body temperature, grip strength (forelimb and hindlimb), and landing foot splay.

5. Motor activity (MA)

Motor activity measurements were assessed for each animal following the FOB observations, prior to exposure and during the 4th, 8th, and 13th, week of treatment. Individual activity was monitored with an Infrared Motion Activity System, (Coulbourn Instruments, Lehigh Valley, PA). MA was measured for 1 hour.

6. Sacrifice/necropsy/neurohistopathology

At study termination, all surviving rats were sacrificed with an anesthetic overdose of sodium pentobarbital (i.p.), and perfused *in situ* with a heparinized flushing agent followed by a 1.5% glutaraldehyde and 4 % paraformaldehyde solution. All animals were examined grossly (external surfaces, orifices, brain, spinal cord, organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck) for lesions when sacrificed. The brain, spinal cord, and nerves, listed in the table below, were examined histologically; 5 rats/sex/group from the control and high concentration groups. Peripheral nerve samples were processed with epon/toluidine blue and a second set was taken for paraffin wax embedding and staining with haematoxylin and eosin. The brain, spinal cord, eyes, optic nerves, skeletal muscle, ganglia, and dorsal and ventral root fibers were embedded in paraffin, then sectioned and stained with haematoxylin and eosin.

X	Brain*	X	Spinal Cord	X	Peripheral nerves
X	Six sections from frontal lobe to medulla (3 forebrain, 1 mid-brain, 1 cerebellum and pons, and 1 medulla oblongata)	X	Cervical - Dorsal root ganglia - Dorsal root fiber - Ventral root fiber - Cervical Swelling - Longitudinal sections	X X	Sciatic nerve, cross and longitudinal Tibial nerve, cross and longitudinal
X	Optic nerves	X	Thoracic Lumbar - Dorsal root ganglion - Dorsal root fiber - Ventral root fiber - Lumbar Swelling	X X X	OTHER Gastrocnemius muscle, right Gasserian ganglion with nerve Both eyes

* Organs that were also weighed

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

There were three mortalities that were attributed to administration of Lindane among females treated at 400/500 ppm in the diet. One female in this group was found dead on Day 11 of the study; this animal suffered weight loss, but no other clinical signs were observed. Another female from this group was found dead during week 10 of the study. This animal had distorted teeth, a swollen muzzle with scabbing, hunched posture, and piloerection prior to death. During Week 13 yet another female was found dead and was observed to have a swollen muzzle with scabbing, hunched posture, and piloerection prior to death. The author surmised that these deaths could have been caused by convulsions. Lindane is reported to produce convulsions and this conclusion is further supported by the finding of blood in the cage of one animal with a distribution which suggested that the animal had flung itself around the cage violently prior to death.

One other female in the 20 ppm group was sacrificed during Week 13 due to the presence of an extra set of incisors which were protruding through the roof of the mouth causing ulcerations in this area. This animal also had staining of the urogenital area and hunched posture.

There were no deaths among male rats in this study.

Other clinical signs that were observed among females treated at the high-dose (400/500 ppm) included, hypersensitivity, staining of the urogenital area, and scabbing on the toes, which may be indicative of self-mutilation.

Clinical signs of toxicity were not observed among females treated at lower doses or among males treated at any concentration of Lindane.

C. BODY WEIGHT AND BODY WEIGHT GAINS

Males and females treated at the high-dose (400/500 ppm) were observed to have significantly lower mean body weights at every weighing performed at the time of FOB assessments, Table 2. At the Week 4 assessment body weights were 12 and 16 % lower compared to the control, at Week 8 they were 10 and 14%, and at Week 13 body weights were 8 and 12 % lower compared to the controls for males and females, respectively.

Body weight gains among males receiving 500 ppm of the test substance were significantly decreased compared to controls for the first week. Females treated at this level also had significantly decreased body weights compared to the control group after one week of treatment at 500 ppm. During the second week of treatment (500 reduced to 400 ppm), recovery was apparent among males and females.

Females treated at 100 ppm also had significantly lower body weight gains compared to the control after one week of treatment. These animals continued to have slight but continuing weight gains compared to the control group.

No effect on body weight gains was observed in males at 100 ppm or lower or among females treated at 20 ppm.

TABLE 2. Mean body weights (g) and body weight gains (g) for male and female rats treated with Lindane for 13 weeks in the diet.								
Day	Group/dose (mg/kg) Lindane							
	0	20	100	500/400	0	20	100	500/400
	Males				Females			
pre-exposure	173	174	175	174	159	159	157	159
4	359	359	350	316**	241	235	224	203**
8	455	454	433	408**	278	276	259	240**
13	523	524	507	483*	304	305	291	267*
Week	Weight gains							
0-1	58	57	55	18**	25	20	15*	-22**
2-3	40	36	34	38	18	15	15	19
2-13	149	145	132	145	59	63	53	67

Data taken from pp. 31 and 45 MRID 44781101.

*p<0.05, **p<0.01

D. FOOD CONSUMPTION AND FOOD CONVERSION RATIOS

Mean food consumption values for the entire study are presented in Table 3. Males and females treated with 500 ppm of the test substance had significantly lower food

consumption compared to controls during the first week of treatment. During the 2nd week, after the 500 ppm dose was reduced to 400 ppm, the males recovered and were consuming quantities of food that were comparable to control values. Recovery among females, however, was not comparable and food intake remained slightly lower compared to controls throughout the study.

Food consumption among females treated at 100 ppm was also significantly reduced compared to control values for the first week of the study. This pattern continued throughout the study.

Males treated at 100 ppm and lower showed normal food consumption throughout the study and females treated at 20 ppm showed no effect compared to controls.

Food conversion ratios were obviously impaired among males and females treated with 500 ppm after the first week of the study as shown in Table 4. Because of the significant body weight loss among females treated at this level, meaningful ratio values could not be calculated. After the high-dose groups were reduced to 400 ppm, ratios were lower in both males and females compared to the control indicating enhanced food conversion ratios for these groups.

TABLE 3. Mean food consumption values for male and female rats treated with Lindane for 13 weeks in the diet.								
Week	Group/dosage (ppm) Lindane							
	Males				Females			
	0	20	100	500/400	0	20	100	500/400
Pre-exposure	183	184	181	184	143	138	137	141
1	200	201	191	131**	151	141	127**	67**
2	200	209	199	176	152	146	139	121
4	197	203	235	190	148	144	135	126**
6	192	201	189	184	145	144	135	124**
10	199	205	195	195	139	136	136	125*
13	193	196	195	201	134	133	135	129

Data taken from p. 32, MRID 44781101.

*p < 0.05, **p < 0.01

TABLE 4. Mean food conversion ratios for male and female rats treated with Lindane in the diet for 13 weeks.

Week	Group/dosage (ppm) Lindane							
	Males				Females			
	0	20	100	500/400	0	20	100	500/400
1	3.5	3.5	3.5	7.4	6.0	6.9	8.5	n/a
2	4.5	4.4	4.5	n/a	7.4	6.8	6.7	n/a
4	7.4	7.3	9.2	6.3	14.6	13.6	19.8	12.2
8	26.2	20.6	19.2	19.8	108.1	32.3	66.9	14.3
10	11.5	13.1	10.7	10.1	16.6	23.8	13.0	15.8
13	85.9	78.9	40.0	25.4	n/a	55.8	68.8	21.4
3-13	10.3	10.8	10.9	9.7	18.8	17.2	18.5	15.3

Data taken from p. 33, MRID 44781101

E. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

Grip Strength

No test substance-related or statistically significant differences in mean forelimb or hindlimb grip strength were observed in any of the treated groups compared to the controls.

Foot Splay

There were no observations of significant differences in mean hindlimb splay between treated and control groups during any of the FOB assessment sessions.

Other FOB Endpoints

Several clinically relevant observations were made among females receiving 500/400 ppm of the test substance. A complete listing of FOB observations (Table A) and rearing activity (Table C) has been appended to this document.

During the Week 4 assessment one female was observed to convulse prior to testing, during testing this animal had an increased respiratory rate. Also during this assessment, three females at this dose (500/400 ppm) were perceived as being significantly more difficult to handle than control animals. The incidences of grooming behavior for females (4 or 5) treated at 100 or 500/400 ppm were increased compared to controls (1) with a significant difference at the high dose only. High-dose females (3) also tended to urinate more in the open arena, and females (5-7) in all treated groups were observed walking on tiptoes with significance obtained in the high-dose group only.

At the Week 8 FOB assessment, females (3) in the 500/400 group were again recorded as being awkward to handle compared to the control. Three females in this group were missing claws and one female as well as two males treated at this dose had hunched posture.

Two males and one female in the high-dose group were observed with piloerection at the Week 13 assessment. Other notable observations in the high-dose group included one female urinating during the pupil reflex test and one female with an unusual head position. Three males and one female treated at the high-dose and one female in the mid-dose (100 ppm) group were awkward to handle.

Additionally, females treated at 500/400 ppm had significantly more activity counts (Table B, appended) than controls at every FOB assessment. At the Week 8 FOB, rearing counts (Table C, appended) were also significantly higher in the high-dose female group compared to the control.

E. MOTOR ACTIVITY

Colburn motor activity was not affected by Lindane administration.

B. NEUROPATHOLOGY

Neuropathological examination revealed no findings that were attributable to the administration of Lindane. Incidental findings were observed with the same frequency in the control and treated groups.

III. DISCUSSION

A. DISCUSSION

This study was initiated with the highest dose set at 500 ppm, however excessive body weight loss, decreased food consumption, and decreased body weight gains prompted the investigators to lower this dose to 400 ppm on Day 11 of the study. The authors attributed this problem to an initial palatability problem as weight loss and failure to gain weight were the only signs of toxicity. However, given the other effects (described below) that were observed in this treatment group it is difficult to attribute the effects on body weight solely to the palatability of the diet preparation.

There were three mortalities among females in the high-dose group that were attributable to the subchronic administration of Lindane in the diet. These animals had exhibited clinical signs prior to death that included hunched posture, swollen muzzles with scabbing, staining of the urogenital region, and piloerection.

Daily observations for clinical signs revealed several test substance-related abnormalities that were confined to females in the highest dose group and included, hypersensitivity to touch, scabbing of the toes, and staining of the urogenital area.

Mean body weight and body weight gain values were affected by exposure to 500/400 ppm Lindane. The mean body weight of males and females treated at this dose was significantly decreased compared to the control group one week after the initiation of treatment. Although these animals did begin to gain weight following the reduction of test substance concentration in the high-dose group, the mean body weights of these animals remained significantly lower than controls throughout the study. Males and females in this group suffered significantly decreased weight gains (70% - 180%) during the first week at a dose of 500 ppm and then a subsequent rebound during the second week was observed after the dose was lowered. Thereafter, weight gains at the 400 ppm dose were reflective of control values. Females in the 100 ppm group also had significantly decreased body weight gains compared to the control group during the first week of the study and this effect continued, although not at a level of significance throughout the remainder of the study. There was also an increase in liver weights noted for both males and females, no further information was provided regarding this observation.

Food consumption was significantly decreased among males and females treated with the 500 ppm of Lindane in the diet during the first week after the initiation of treatment. However, during the 2nd week, food consumption values were comparable to or exceeded control values among males. Females did not exhibit this same level of recovery as their food consumption remained slightly depressed throughout the remainder of the study. This effect was clearly treatment-related. Females in the 100 ppm group had significantly decreased food consumption for the first week of the study and this trend continued throughout the study although not at a level of significance.

Among animals treated with 500 ppm of the test substance a significant effect on food conversion ratios was evident after one week of administration. This effect was eliminated after the dose was lowered to 400 ppm.

Several neurotoxic endpoints were observed from the FOB assessments in this study that were regarded as treatment-related. These were also mainly confined to the highest dose group although some effects were observed among females treated at 100 ppm. Throughout the study, both males and females treated at the highest dose (500/400 ppm) were perceived as difficult to handle; however, only females had higher motor activity counts. In week 4, females (5-7) across the dose groups were observed walking on tiptoes and these incidences were significantly increased compared to the control for the highest dose group. During this same assessment period, six females in the 500/400 ppm dose group tended to urinate more often than controls and one female was observed to convulse; in the 100 ppm group, five females had increased incidences of grooming behavior and one animal in this group was extremely difficult to handle. At the week 8 assessment, animals in the highest dose group were observed to have hunched posture (2♂, 1♀). Females in this group also had missing claws (3), and higher incidence rearing. There were two males and one female treated at 500/400 ppm with piloerection at 13 weeks.

The assessments of forelimb and hindlimb grip strength as well as hindlimb splay revealed no differences for any of the treated groups compared to the control groups.

Colburn motor activity was also similar among treated groups compared to the control groups.

Neurohistopathology examinations did not indicate any exposure-related effects. The investigators did see a significant increase in liver weight compared to the controls among animals treated at 500/400 ppm.

The NOAEL for systemic toxicity is 100 ppm for males (7.1 mg/kg) and 20 ppm for females (1.6 mg/kg). Based on the substance-related effects on body weight, body weight gain, food consumption, and increased liver weight (500/400 ppm only). The LOAEL levels for systemic toxicity in males is 500/400 ppm (28.1 mg/kg) and 100 ppm for females (7.9 mg/kg).

The NOAEL for neurotoxic effects is 100 ppm for males (7.1 mg/kg) and females (7.9 mg/kg). The neurotoxicity LOAEL is 500/400 ppm based on hypersensitivity to touch and hunched posture.

B. STUDY DEFICIENCIES

None

LINDANE

Subchronic Oral Neurotoxicity (82-7)

TABLE A
SUMMARY OF FUNCTIONAL OBSERVATIONAL BATTERY

Pre-dose

	Males				Females			
Group	1	2	3	4	1	2	3	4
No. of animals	10	10	10	10	10	10	10	10
OBSERVATIONS:								
HOME CAGE								
posture = s/r	9	10	10	10	10	10	10	10
REMOVAL FROM CAGE								
removing, easy	9	8	10	9	10	9	10	10
handling, easy	10	9	7	8	10	9	7	9
Salivation	0	0	0	0	0	0	0	0
Vocalising	0	1	1	1	0	2	2	2
IN THE ARENA								
Tremors	1	1	1	0	0	1	0	0
Grooming	4	4	3	5	1	2	1	1
arousal, alert	10	6	6	9	10	10	10	10
Defecation	6	6	3	5	1	1	1	0
Urine	3	4	2	2	2	0	3	1
GAIT								
walking on toes	1	2	1	1	0	3	0	5
Hunched	0	0	0	0	0	1	0	0
Unsteady	0	0	0	0	6	1?	0	0
unable to assess	1	4	3	0	0	0	0	0
MANIPULATIONS								
approach, a reaction	10	10	10	10	10	10	10	10
touch, a reaction	8	9	10	10	8	9	10	9
startle (present)	10	10	10	10	10	10	10	10
righting, immediately	10	10	10	10	10	10	10	10
tail pinch, a reaction	10	10	10	10	10	10	10	10
pupil reflex	10	10	10	10	10	10	10	10

s/r = sitting/rearing in cage

Numbers reflect the number of animals showing the response or with the indicated score

TABLE A
(Summary of functional observational battery - continued)

		Week 4							
		Males				Females			
Group		1	2	3	4	1	2	3	4
No. of animals		10	10	10	10	10	10	10	9
OBSERVATIONS:									
HOME CAGE									
posture = s/r		10	10	10	10	10	10	10	9
REMOVAL FROM CAGE									
removing, easy		10	10	10	10	9	10	9	8
handling, easy		10	9	10	10	10	10	9	7a
salivation		1	0	0	0	0	0	0	0
vocalising		2	0	2	1	1	2	1	3
IN THE ARENA									
tremors		2	2	3	0	0	0	0	0
piloerection		0	0	0	0	0	0	0	1
grooming		3	1	3	3	1	2	4	5b
arousal, alert		6	5	6	10	10	10	9	9
defecation		2	5	1	2	0	0	0	1
urine		2	4	3	3	1	0	0	3
GAIT									
walking on toes		2	0	0	0	1	7	5	5c
hunched		0	0	0	0	0	2	0	0
unable to assess		3	4	4	1	0	0	1	0
MANIPULATIONS									
approach, a reaction		10	8	10	10	10	10	10	9
touch, a reaction		8	6	8	5	10	8	9	9
startle (present)		10	10	10	10	10	10	10	9
righting, immediately		10	10	10	10	10	10	10	9
tail pinch, a reaction		10	10	10	10	10	10	9	9
pupil reflex		10	10	10	10	10	10	10	9

s/r = sitting/rearing in cage

Numbers reflect the number of animals showing the response

a, $p = 0.049$

b, $p = 0.026$

c, $p = 0.048$

TABLE A
(Summary of functional observational battery - continued)

Week 8

Group No. of animals	Males				Females			
	1	2	3	4	1	2	3	4
	10	10	10	10	10	10	10	9
OBSERVATIONS:								
HOME CAGE								
posture = s/r	10	10	10	10	10	10	10	9
REMOVAL FROM CAGE								
removing, easy	10	10	10	10	10	10	10	9
handling, easy	9	10	7	9	9	8	8	7
Salivation	1	2	1	2	0	0	0	0
vocalising	4	2	2	3	3	2	1	3
IN THE ARENA								
Tremors	4	4	1	2	2	0	0	0
Grooming	1	2	3	1	2	3	3	3
pilo erection	1	0	0	0	0	0	0	0
arousal, alert	7	6	5	8	10	10	9	9
Defecation	0	5	2	2	0	0	0	1
Urine	3	6	5	6	1	1	1	2
GAIT								
walking on toes	2	3	2	2	6	7	3	6
Swaying	1	0	0	0	0	1	0	1
hunched	0	0	0	2	0	0	0	1
unable to assess	5	5	4	2	0	0	1	0
MANIPULATIONS								
approach, a reaction	9	10	9	10	10	10	10	9
touch, a reaction	8	3	7	8	9	8	8	8
startle (present)	10	10	10	10	10	10	10	9
righting, immediately	9	9	10	10	10	10	10	9
tail pinch, a reaction	10	10	10	10	10	10	10	9
pupil reflex	10	10	10	10	10	10	10	9
ADDITIONAL COMMENTS								
missing claws	0	0	0	0	0	0	0	3a

s/r = sitting/rearing in cage

Numbers reflect the number of animals showing the response

a, p = 0.009

TABLE A
(Summary of functional observational battery - continued)

Week 13

Group No. of animals	Males				Females			
	1	2	3	4	1	2	3	4
	10	10	10	10	10	9	10	7
OBSERVATIONS:								
HOME CAGE								
posture = s/r	10	10	10	10	10	9	10	7
REMOVAL FROM CAGE								
removing, easy	9	10	10	10	10	9	10	7
handling, easy	10	9	10	10	6	9	8	6
pilo-erection	0	0	0	0	0	0	0	1
salivation	1	0	0	0	0	1	0	1
vocalising	4	2	2	1	4	1	1	1
IN THE ARENA								
tremors	3	2	3	3	1	0	1	0
piloerection	0	0	0	2	0	0	0	0
grooming	0	1	4	1	3	2	0	2
arousal, alert	6	7	8	8	10	9	9	6
defecation	0	1	0	1	0	0	0	0
urine	2	3	2	3	1	3	2	1
GAIT								
walking on toes	2	3	1	1	6	8	7	5
hunched posture	0	0	0	1	0	0	0	0
unable to assess	4	4	3	2	0	0	1	0
MANIPULATIONS								
approach, a reaction	8	10	9	9	10	9	9	7
touch, a reaction	6	9	6	7	8	7	10	6
startle (present)	10	10	10	10	10	9	10	7
righting, immediately	10	10	9	10	10	9	10	7
tail pinch, a reaction	10	10	10	10	10	8	10	7
pupil reflex	10	10	10	10	10	9	10	7
ADDITIONAL COMMENTS								
wet urogenital region	0	0	0	0	0	0	0	1
moderately awkward to handle	0	0	0	3a	0	1	0	0
extremely awkward to handle	0	0	0	0	0	0	1	1
unusual head position	0	0	0	0	0	0	0	1

s/r = sitting/rearing in cage

Numbers reflect the number of animals showing the response

a, p = 0.012

TABLE B
Activity counts - group mean values

Pre-dose

Group/dosage (ppm)	Mean activity counts			
	Males	sd	Females	sd
1-0	11	6.2	20	6.1
2-20	11	7.8	16	8.7
3-100	9	7.5	17	5.7
4 -400/500	14	6.2	23	6.6

Week 4

Group/dosage (ppm)	Mean activity counts			
	Males	sd	Females	sd
1-0	13	9.9	18	8.6
2-20	9	7.5	23	3.6
3-100	10	8.5	15	10.8
4 -400/500	18	7.3	27*	9.2

Week 8

Group/dosage (ppm)	Mean activity counts			
	Males	sd	Females	sd
1-0 12	11.5	16	7.4	
2-20	10	8.4	20	5.0
3-100	9	10.8	19	8.1
4-400/500	15	8.9	32**	12.7

Week 13

Group/dosage (ppm)	Mean activity counts			
	Males	sd	Females	sd
1-0	12	11.0	23	10.4
2-20	10	7.5	25	6.1
3-100	11	9.5	27	10.6
4 -400/500	15	10.1	33*	10.4

sd Standard deviation

* p<0.05, ** p<0.01

TABLE C

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LINDANE**Subchronic Oral Neurotoxicity (82-7)****Rearing counts - group mean values****Pre-dose**

Group/dosage (ppm)	Mean rearing counts			
	Males	sd	Females	sd
1-0	6	3.1	11	5.7
2-20	6	6.0	9	4.9
3-100	4	4.6	9	3.3
4 -400/500	8	3.4	9	3.4

Week 4

Group/dosage (ppm)	Mean rearing counts			
	Males	sd	Females	sd
1-0	7	6.0	10	4.5
2-20	5	4.9	11	4.2
3-100	6	6.4	8	5.4
4-400/500	12	5.2	14	7.3

Week 8

Group/dosage (ppm)	Mean rearing counts			
	Males	sd	Females	sd
1-0	6	4.7	7	4.7
2-20	6	5.5	9	3.6
3-100	5	6.1	8	4.5
4 - 400/500	8	6.6	20**	10.7

Week 13

Group/dosage (ppm)	Mean rearing counts			
	Males	sd	Females	sd
1-0	5	4.4	12	5.7
2-20	5	4.7	12	4.2
3-100	5	4.0	11	5.1
4-400/500	8	5.8	15	4.2

sd = Standard deviation

**p<0.01

LINDANE

Subchronic Oral Neurotoxicity (82-7)

SignOff Date:	8/2/00
DP Barcode:	D254526
HED DOC Number:	014276
Toxicology Branch:	CEB2