(8/2/00)

014274

DATA EVALUATION REPORT

LINDANE
(gamma HCH)

STUDY TYPE: ACUTE ORAL (GAVAGE) NEUROTOXICITY – RAT (81-8)

MRID 44769201

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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Reregistration Branch 4 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral (Gavage) Neurotoxicity – Rat
OPPTS 870.6200 [§81-8]

DP BARCODE: D254122
P.C. CODE: 009001
MRID: 44769201

SUBMISSION CODE: S558248
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. Neurotoxicity study by a single oral gavage administration to CD rats followed by a 14-day observation period. Huntingdon Life Sciences Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE186ES, England. Project Identity CIL/011 February 25, 1999, MRID 44769201, Unpublished.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 an acute oral neurotoxicity study (MRID 44769201), groups of 10 Crl:CD®BR rats/sex/dose were administered single dose of Lindane (Batch No. HLS96/1, Purity 99.78%) by gavage at concentrations of 0 (control), 6, 20, or 60 mg/kg. Functional observational battery (FOB) and motor activity (MA) testing were performed prior to administration and within 3 hours (time of peak effect) of dosing (day 0), and on days 7 and 14 post-dose. 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, designated tissues of the nervous system were processed for microscopic neuropathological evaluation.

All animals survived to scheduled termination. One male in the 60 mg/kg group was observed to convulse on the day of treatment within 2.75 hours after dosing. Clinical signs were also observed in females treated at 60 mg/kg within 24 hours of dosing and included: staining of the fur, stained urogenital region, hunched posture, and piloerection. These effects in females persisted for four days. Significant treatment-related decreases in body weight gains were observed for males in the 60 mg/kg group compared to the control group for the first week of the study. Females administered this concentration also had slightly lower body weight gains

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throughout the study. Food consumption for males and females administered 60 mg/kg was significantly decreased compared to controls for Week 1 of the study. Food conversion ratios in the treated groups were not changed compared to control groups.

At the first FOB assessment on Day 0 (3 hours after dosing) males and females in the 60 mg/kg group exhibited piloerection (1 ♂, 2 ♀), decreased rectal temperature (1 ♂, 1 ♀), increased hindlimb foot splay and hunched posture (4 ♂, 7 ♀). Among males dosed at 60 mg/kg, increased respiration (3 ♂, 1 ♀) and one observation of tremor/twitching were observed. Females administered 60 mg/kg were observed to have increased incidences of walking on tip toes (10), licking behavior (3), decreased foot splay (3) and an absence of grooming (8) behavior. Females in the 20 mg/kg also had decreased grooming (3) behavior and increased forelimb grip strength. Motor activity was significantly decreased for males and females treated with 60 mg/kg as well as among females treated with 20 mg/kg three hours post-treatment. The 6 mg/kg group remained comparable to controls in FOB assessment parameters and MA.

No neuropathological endpoints 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 20 mg/kg for males and 6 mg/kg for females. Based on the substance-related effects on body weight, body weight gain, food consumption, and clinical signs of toxicity the LOAEL for systemic toxicity in males is 60 mg/kg. The LOAEL for females is 20 mg/kg based on a lower incidence of grooming behavior and decreased locomotor activity immediately after dosing, in addition to the parameters mentioned above.

The NOAEL for neurotoxic effects is 6 mg/kg for females and the LOAEL is 20 mg/kg based on increased forelimb grip strength and decreased grooming behavior and motor activity (MA). The NOAEL for neurotoxicity in males is 20 mg/kg and the LOAEL for males is 60 mg/kg based on tremors, convulsions, decreased MA, and increased forelimb grip strength.

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

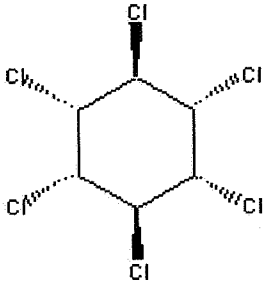
A. MATERIALS

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:

http://www.chemfinder.com



2. Vehicle

Corn oil, (maize oil BP)

3. Test animals

Species: rat
Strain: Crl:CD®BR
Age and mean weight at study initiation: 35 day old males within 34 g weight range and 28 day old females within 16 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*, except overnight fast prior to dosing.
Water: Filtered tap water was available *ad libitum* from an automatic watering system.

Environmental conditions:
Temperature: 20±0.5 °C
Humidity: 50±10%
Air changes: not given
Photoperiod: 12 hr light/12 hr dark
Acclimation period: at least 12 days for males and 19 days for females

B. STUDY DESIGN

1. In life dates

Start: May 4, 1998
End: May 29, 1998

2. Animal assignment

All animals were received on April 22, 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	Dose (mg/kg) Lindane	Number of animals	
		Males	Females
1. White	0.00 (control)	10	10
2. Yellow	6.00	10	10
3. Blue	20.00	10	10
4. Pink	60.00	10	10

Data taken from pp. 15, MRID 44769201.

3. Validation of test methods

A report entitled "Acute Neurotoxicity Study Validation Using Positive Controls" was submitted on April 26, 1999 by CIEL (MRID 44811401). The report contains two manuscripts which describe the neurotoxic effects of trimethyltin chloride, acrylamide, carbaryl and p,p'-DDT.

4. Rationale for dose selection

The doses selected for this study were based on a dose range-finding study performed by Huntingdon Life Sciences (CIL 015/982938).

5. Preparation, administration, and analysis of test suspensions

The test substance was administered as a suspension in corn oil by oral gavage. The test material was ground with a mortar and a small amount of the vehicle until a smooth paste was formed. The suspension was formed by gradually adding and mixing the vehicle until the desired concentrations were achieved. The formulations were then mixed in a high shear homogenizer.

Results

Proximity to Target. Samples of specimen corn oil formulations (400 mL) which contained lindane at nominal concentrations of 0.2 mg/ml and 20 mg/ml were subdivided into four 100 mL samples each and either analyzed immediately, after 2 days at room temperature, or after 2, 8, and 15 days at 4°C. The mean concentrations of the test formulations were all found to be within -7% of nominal concentrations.

Conclusion. These analyses confirm that the dosing suspensions were within acceptable proximity to target concentrations each day of sampling, over each dosing concentration, and that the suspensions were stable (at least 15 days) over the dosing period.

6. Estimated time of peak effect

Time to peak effect was estimated at 3 hours post-dosing based on a dose range-finding study performed by Huntingdon Life Sciences (CIL 015/982938).

7. Statistical analysis

Statistical analyses were carried out separately for males and females.

Many of the parameters in this study were adjusted for pre-dose values as covariate.

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.

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, and once a week thereafter.

3. Food consumption and food conversion ratios

Food consumption and food conversion ratios were determined weekly.

$$\text{Food consumption (g/rat/week)} = \frac{\text{Total food given - Total food left}}{\text{\# of animal days*}} \times 7$$

$$\text{Food conversion ratio} = \frac{\text{Food consumed}}{\text{Body weight gain}}$$

* 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 on days 0 (at time of peak effect-three hours post-treatment), 7, and 14 post-treatment.

a. Home Cage Observations

Animals were observed in their closed home cages for posture, tremor, twitches, spontaneous vocalizations, 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, 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 on days 0 (3 hours post-dose), 7 and 14 post-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 (day 15), all 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 (0 mg/kg) and high concentration (60 mg/kg) groups. Peripheral nerves 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	X	Cervical	X	Sciatic nerve, cross and longitudinal
			- Dorsal root ganglia	X	Tibial nerve, cross and longitudinal
X	Optic nerves		- Dorsal root fiber		
			- Ventral root fiber		Other
			- Cervical Swelling		
			- Longitudinal sections	X	Gastrocnemius muscle, right
			Thoracic	X	Gasserian ganglion with nerve
		X	Lumbar	X	Both eyes
			- Dorsal root ganglion		
			- Dorsal root fiber		
			- Ventral root fiber		
			- Lumbar Swelling		

* Organs that were also weighed

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

All animals survived to termination of the study. Within 2.75 hrs of dosing, one male in the 60 mg/kg group was observed to be convulsing. Twenty-four hrs after dosing, females treated with 60 mg/kg were observed to have staining of the urogenital region, staining of the fur, hunched posture, and piloerection. The hunched posture and piloerection were resolved within 3 days after treatment, the other symptoms persisted considerably longer.

Daily cage observations and weekly physical examinations revealed no substance-related abnormalities in the lower exposure groups.

C. BODY WEIGHT AND BODY WEIGHT GAINS

Body weights among males treated at 60 mg/kg were significantly lower than controls on day 8 of the observation period (Table 3). Females receiving 60 mg/kg of the test substance had significantly lower body weights compared to controls on day 15 of the study.

Body weight gains among males receiving 60 mg/kg of the test substance were significantly decreased compared to controls for the first week following dosing. Females treated at this level also had slightly decreased body weight gains, but these differences were not significantly different from the control group. During the second week of the observation period, the males recovered and exceeded the controls. Body weight gains among females however, remained slightly lower than controls such that cumulative weight gains for the entire observation period were significantly lower than controls.

No effect on body weight gains was observed in animals treated at lower doses.

Table 3. Mean body weights (g) and body weight gains (g) for male and female rats treated with Lindane by oral gavage.

Day	Group/dose (mg/kg) Lindane							
	0	6	20	60	0	6	20	60
	Males				Females			
pre-exposure	241	237	235	235	167	173	171	168
1	212	209	208	208	148	153	150	149
8	285	280	280	272*	191	191	193	187
15	325	321	323	323	214	213	211	206**
Week	Weight gains							
0-1	79	74	75	66*	45	46	48	41
1-2	40	40	41	52**	23	21	17	19
0-2	119	115	116	118	68	67	65	60*

Data taken from p. 38 and p. 51 MRID 44769201
*p<0.05, **p<0.01
Values were adjusted for pre-dose as covariate

D. FOOD CONSUMPTION AND FOOD CONVERSION RATIOS

Mean food consumption values for the entire study are presented in Table 4. Males and females treated with 60 mg/kg of the test substance had significantly lower food consumption compared to controls during the first week of the observation period after treatment. During the 2nd week, these animals recovered and were consuming quantities of food that were comparable to or exceeded control values.

Food consumption in other exposure groups remained comparable to control values throughout the study.

Table 4. Mean food consumption values for male and female rats treated with Lindane by oral gavage.‡

Week	Group/dosage (mg/kg) Lindane							
	Males				Females			
	0	6	20	60	0	6	20	60
Pre-exposure	184	177	183	184	133	140	135	133
1	221	214	217	194**	171	175	172	152**
2	228	230	230	241	167	172	168	166

Data taken from p. 39, MRID 44769201.
**p<0.01
‡Food consumption measured over 6 days as animals starved overnight prior to dosing.

E. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

Grip Strength

No test substance-related or statistically significant differences in mean hindlimb grip strength were observed in any of the test concentration groups either in males or females. Forelimb grip strength was significantly increased for high-exposure males and females as well as for females exposed to 20 mg/kg on day 1, three hours after dosing (Table 5). Forelimb grip strength was normal on the other assessment days and at lower test concentrations.

Table 5. Summary of mean forelimb grip strength (kg) in rats administered Lindane by oral gavage.

Day of Assessment	Exposure concentration (mg/kg)			
	0	6	20	60
Males				
Pre-exposure	0.75	0.77	0.79	0.68
Day 1	0.83	0.94	0.89	0.98**
Females				
Pretest	0.62	0.71	0.63	0.74*
Day 1	0.73	0.78	0.83**	0.90**

Values were adjusted for pre-dose as covariate.
Data taken from p. 47 (MRID 44769201); * $p < 0.05$, ** $p < 0.01$

Foot Splay

Landing footsplay was significantly decreased (12%), when adjusted for pre-dose as co-variate, among females treated at 60 mg/kg compared to controls on day 1 (3 hrs after dosing). There were no substance-related statistically significant differences for males or females at any of the lower test concentrations for mean hindlimb foot splay during the exposure period.

Other FOB Endpoints

Three hours post-dosing, several clinically relevant observations were made among males and females receiving 60 mg/kg of the test substance. Males and females in the 60 mg/kg group exhibited: piloerection (1 ♂, 2 ♀), hunched posture (4 ♂, 7 ♀), increased hindlimb foot splay (females only) and decreased mean rectal temperature. Among males dosed at 60 mg/kg, increased respiration (3) and one observation of tremor/twitching were observed. Females administered 60 mg/kg were observed to have increased incidences of walking on tip toes (6), licking behavior (3), and vocalizations(3). Significantly decreased grooming behavior was observed among

females treated at 60 and 20 mg/kg. One male and one female in the 60 mg/kg group and one female in the 20 mg/kg group felt cold to the touch.

On day 8 after dosing, females in the 60 mg/kg group were still observed walking on tiptoes (3) and males in this group had an increased incidence of salivation (4).

E. MOTOR ACTIVITY

At 3 hrs post-dosing, males and females that received 60 mg/kg had significantly decreased locomotor activity compared to the controls, (Table 6). Other treatment groups were comparable to the control group.

On day 8 there were no significant differences observed among any of the treated groups compared to controls.

During the MA assessment on day 15, males in the 60 mg/kg group had an increased level of activity compared to the control group, (Table 6). No effects were observed in the lower dosage groups.

Table 6. Mean motor activity (in secs) during a one hour session for male and female rats treated with Lindane by oral gavage.

Day	Group/dose (mg/kg) Lindane							
	Males				Females			
	0	6	20	60	0	6	20	60
pre-exposure	656	698	516	697	623	753	788	737
1	167	295	160	85	348	444	320	131**
8	698	830	592	932	467	488	548	443
15	645	779	656	907*	556	631	575	537

Data taken from pp. 52-54, MRID 44769201 (*p<0.05, **p<0.01)

B. NEUROPATHOLOGY

There were no observations of treatment-related neuropathological findings in any of the exposure groups for either sex. There were some observations of axonal degeneration in some peripheral nerves and spinal nerve roots/spinal cord, however these were sporadic, occurred across dose groups, and were considered normal background findings. Retinal folds were also observed in several control animals and in one animal treated at 60 mg/kg.

III. DISCUSSION

A. DISCUSSION

There was no mortality among males or females in any of the test groups after acute oral exposure to Lindane. Clinical observations revealed several test substance-related abnormalities that were mainly confined to the highest dose group, were evident immediately after dosing, and decreased with respect to incidence and severity thereafter. These observations included: convulsions in one male and tremors in another male, both in the 60 mg/kg; staining of the urogenital area among females in the 60 mg/kg group; and hunched posture and piloerection in males and females treated with 60 mg/kg.

Mean body weight and body weight gain values were affected by exposure to 60 mg/kg Lindane. The mean body weight of males treated at this dose was significantly decreased compared to the control group one week after dosing and the mean body weight of females was significantly decreased compared to controls on day 15 of the study. Mean body weight gains were similarly affected among animals treated at this dose. Males in this group suffered significantly decreased weight gains during the first week following the acute dose and then a subsequent rebound during the second week of the observation period. Females suffered a cumulative decrease in weight gain for the entire observation period compared to the control group. These effects were considered to be treatment-related.

Food consumption was significantly decreased among males and females treated with the 60 mg/kg dose of Lindane during the first week of the observation period. This effect was also treatment-related despite the absence of a significant effect on food conversion ratios in this study. However, during the 2nd week, food consumption values were comparable to or exceeded control values.

Several neurotoxic endpoints were observed from the FOB assessments in this study, these were also mainly confined to the highest dose group although some effects were observed among females treated at 20 mg/kg. Piloerection (1 ♂, 2 ♀), tremors/twitching (1 ♂), increased urination (3 ♂, 3 ♀), hunched posture (4 ♂, 7 ♀), vocalization upon handling (1 ♂, 3 ♀), increased respiration (3 ♂, 1 ♀), and licking (1 ♂, 3 ♀) were observed with increased incidence among males and females in the 60 mg/kg group in the open arena. One male and one female in the 60 mg/kg group, and one female in the 20 mg/kg group were cold to the touch. Grooming behavior was notably decreased among females in the 20 and 60 mg/kg groups, and six females treated at 60 mg/kg were observed walking on toes to a moderate degree. These observations were typically confined to the assessment conducted just after dosing (3 hrs), except for the tiptoe walking in females which persisted through the day 8 assessment. Mean rectal temperature was significantly decreased compared to the control group among males and females in the 60 mg/kg group on the day of dosing. This effect was also observed among males only at the day 8 assessment.

Forelimb grip strength was also affected by acute administration of Lindane within 3 hrs of the exposure. Males and females receiving 60 mg/kg of the test substance as well as females in the 20 mg/kg group had significantly increased forelimb grip strength compared to controls. Foot splay was significantly decreased among females in the 60 mg/kg group. This effect was not observed at any of the other neurobehavioral assessments.

Treatment-related effects on motor activity were observed among males and females in the 60 mg/kg treatment group on the day of dosing. Less locomotor activity was observed among these animals during the neurobehavioral assessment conducted 3 hrs after dosing. Locomotor activity was increased among males treated at 60 mg/kg on day 15 of the study with no similar effect in females at this time point. The relevance of this increased effect on motor activity is dubious at best due to the assertion made by the authors that when the 1 hr MA assessment was analyzed by 10-min blocks, only the first 10-min period actually showed a significant effect. This was similar to the pattern observed in the control group.

Neurohistopathology examinations did not indicate any exposure-related effects.

The authors chose to adjust the post-dose values for many of the parameters to reflect the pre-dose values of each animal. Thus the individual animal was considered as the basic experimental unit. It is the reviewer's opinion that in most cases this type of consideration allows for a more sensitive assessment of neurobehavioral effects and did not significantly alter the conclusions in this study.

The NOAEL for systemic toxicity is 20 mg/kg for males and 6 mg/kg for females. Based on the substance-related effects on body weight, body weight gain, food consumption, and clinical signs of toxicity the LOAEL for systemic toxicity in males is 60 mg/kg. The LOAEL for females is 20 mg/kg based on a lower incidence of grooming behavior and decreased locomotor activity immediately after dosing, in addition to the parameters mentioned above.

The NOAEL for neurotoxic effects is 6 mg/kg for females and the LOAEL is 20 mg/kg based on increased forelimb grip strength and decreased grooming behavior and MA. The NOAEL for neurotoxicity in males is 20 mg/kg and the LOAEL for males is 60 mg/kg based on tremors, convulsions, decreased MA, and increased forelimb grip strength.

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

B. STUDY DEFICIENCIES

No study deficiencies were identified.

SignOff Date:	8/2/00
DP Barcode:	D254122
HED DOC Number:	014274
Toxicology Branch:	CEB2