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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA ID#: 009001. Lindane: Review of rat and rabbit developmental toxicity studies conducted in 1971 and 1976.

TOX CHEM No.: 527
PC No.: 009001
Barcode No.: D190328 and D190460
Submission No.: S438935 and 438961

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THROUGH: Marion Copley, DVM, Section Head
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Health Effects Division (H7509C) 7/8/93

I. CONCLUSION

The two rat series 83-3 developmental toxicity studies (oral administration, Huntingdon # 4307/71/463, Dec 3, 1971 and subcutaneous administration, Hazleton # 405-104, July 13, 1976) were combined and determined to be CORE MINIMUM data to satisfy the requirement for a series 83-3 developmental toxicity study in rats. The combined data support a maternal toxicity NOEL and LEL of 5 and 10 mg/kg/day based on decreased body weight and feed consumption and a developmental toxicity NOEL and LEL of 10 and 20 mg/kg/day based on the presence of "extra ribs" in one strain (CFY) of rat.

The combination of the oral (Huntingdon # 4308/71/64, Dec. 2, 1971) and subcutaneous (Hazleton # 405-103, August 6, 1976) rabbit developmental toxicity studies were also considered CORE MINIMUM data. The data support a NOEL/LEL of > 20 mg/kg/day

for both maternal and developmental toxicity.

No additional rat or rabbit series 83-3 developmental toxicity studies are required at this time. the HED RfD Committee has concurred with this conclusion.

II. Background

As a part of the reregistration of lindane, a survey of the toxicity data base in HED files could not locate DERs for the developmental toxicity studies (series 83-3) in rats or rabbits. In order to update the files, these studies were requested from and later provided by the Reregistration Division. These studies were reviewed by Toxicology Branch I (TB-I) and the DERs are attached. The following comments apply.

III. Toxicology Branch Comments

A. Rat Studies.

1. Both rat studies (oral administration, Huntingdon Laboratories Study No.: 4307/71/463, December 3, 1971 and subcutaneous administration, Hazleton Laboratories Study No.: 404-104, July 13, 1976) were combined such that the oral study was determined to be CORE MINIMUM.

2. These studies when taken together support the position that the CFY rat has a NOEL and LEL for maternal toxicity of 5 and 10 mg/kg/day with decreases in body weight gain and food consumption and at higher dose levels (20 mg/kg/day) death results. Reduced body weight and feed consumption (at 15 mg/kg/day and above) and deaths (at 30 mg/kg/day) were also the only symptoms noted in Sprague-Dawley strain rat following subcutaneous dosing.

The NOEL and LEL for developmental toxicity in the rat is set at 10 and 20 mg/kg/day based on the presence of "extra ribs" and total pups with skeletal variants at the dose level of 20 mg/kg/day in the CFY strain rat. A trend or this condition was also noted in the lower two dose levels. There was, however, no evidence of "extra ribs" in the Sprague-Dawley strain rat at the dose level of 30 mg/kg/day following subcutaneous administration of lindane.

3. No additional series 83-3 developmental toxicity studies in rats are required at this time.

B. Rabbit Studies

1. The first study (oral administration, Huntingdon Laboratories, Study No.: 4308/71/64, Dec. 2, 1971) did not demonstrate any toxicity at any dose level and the highest dose level was 20 mg/kg/day. The second study (subcutaneous adminstration, Hazleton Laboratories, Study No.: 405-103, August 6, 1976) demonstrated indications of maternal toxciity but no fetal toxicity. Although signs of maternal toxicity in rabbits by the subcutaneous route of administration were evident at 15 mg/kg/day, this route of compound administration is not considered applicable to human health risk assessment. Both studies were combined such that the oral study can be classified as CORE MINIMUM.

2. Data from the subcutaneous rabbit developmental toxicity study (Hazleton #405-103, August 6, 1976), however, help to support the dose selection and the study can be upgraded to MINIMUM. Subcutaneously administered lindane was demonstrated to cause significant maternal toxicity at a dose level of 15 mg/kg/day. No evidence of developmental toxicity in rabbits was evident at the highest dose level (15 mg/kg/day) which had fetuses available for evaluation. In the rat, there was no developmental toxicity at the maternal LEL for either the oral (10 mg/kg/day) or subcutaneous (15 mg/kg/day) route of exposure. Therefore, testing at dose levels in rabbits higher than 20 mg/kg day would not be expected to alter the conclusions regarding developmental toxicity.

3. These two studies when taken together support the position that the rabbit has a NOEL > 20 mg/kg/day for both maternal and developmental toxicity following oral administration. These assignments for NOEL are higher than the NOEL/LEL for maternal toxicity and developmental toxicity for the rat (refer to item A2 above).

4. No additional series 83-3 developmental toxicity studies are required in rabbits at this time.

iV. Studies Reviewed.

Study Identification	Material	MRID No.:	Results	Classification
83-3. Developmental Toxicity-rats (oral). Huntingdon Research Centre Study # 4307/71/463, December 3, 1971	Lindane Batch # 6801/403	428680-01	Maternal Toxicity NOEL/LEL: 5/10 mg/kg/day. Body weight gain decrease and food consumption decrease. At 20 mg/kg/day: Deaths. Developmental Toxicity NOEL/LEL: 10/20 mg/kg/day. Extra ribs. CFY strain rat. Dose levels tested 0, 5, 10, and 20 mg/kg/day in 0.5% carboxymethylcellulose.	MINIMUM
83-3. Developmental Toxicity-rats ~~(oral)~~ subcutaneous Hazleton America, Study No.: 405-104, July 13, 1976	Lindane Lot # 36346	00062658	Maternal Toxicity NOEL/LEL: 5/15 mg/kg/day. Body weight and food consumption decreases. At 30 mg/kg/day; Deaths. Developmental Toxicity NOEL/LEL: > 30 mg/kg/day. Sprague-Dawley Strain rat. Dose levels tested 0, 5, 15 and 30 mg/kg/day in corn oil.	SUPPLEMENTARY
83-3. Developmental Toxicity-rabbits (oral) Huntingdon Research Centre Study No.: 4308/71/64 December 2, 1971	Lindane Batch No.: 6801/403	428680-02	Maternal Toxicity and Developmental Toxicity NOEL > 20 mg/kg/day. *Classification is when study is combined with the subcutaneous administration study below. New Zealand White rabbits; Dose levels tested: 0, 5, 10, and 20 mg/kg/day in 0.5% carboxymethylcellulose.	MINIMUM

83-3. Developmental Toxicity-rabbits (subcutaneously) Hazleton America, Study No.: 405-103. August 6, 1976	Lindane Batch No.: not stated.	00062653	Maternal Toxicity NOEL/LEL: 5/15 mg/kg/day. Death and clinical signs, decreased body weight and food consumption, changes in appearance of the liver. At 45/30 mg/kg/day: Deaths and discontinuance of group from developmental toxicity assessment. Developmental Toxicity NOEL/LEL: > 15 mg/kg/day. New Zealand White rabbits. Dose levels tested: 0, 5, 15 and 45/30 mg/kg/day in corn oil.	SUPPLEMENTARY

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Reviewed by: John Doherty John Doherty 6/11/93
Section IV, Toxicology Branch I (H7509C)
Secondary reviewer: Marion Copley, DVM
Section IV, Toxicology Branch I (H7509C) Marion Copley 6/10/93

DATA EVALUATION REPORT

STUDY TYPE: 83-3. Developmental Toxicity - Rats (subcutaneous)

MRID NO.: 00062656 (Book) TOX. CHEM. NO.: 527
PC No.: 009001

TEST MATERIAL: Technical lindane (gamma "benzene hexachloride, USP") from lot No.: 36346 described as a white granular material with an unpleasant odor.

STUDY NUMBER(S): 405-104

SPONSOR: Reed and Carnrick Research Institute

TESTING FACILITY: Hazleton Laboratories, America

TITLE OF REPORT: "Teratology Study in Rats Lindane (Gamma Benzene Hexachloride, USP)"

AUTHOR(S): Frederick F. Reno (Director of Toxicology Dept.)

REPORT ISSUED: July 13, 1976

STUDY DATES: Not specified.

CONCLUSIONS:

NOEL and LEL (maternal toxicity) = 5 and 15 mg/kg/day. Decreased body weight. At 30 mg/kg/day: tremors, convulsions and death (2 dams).

NOEL (developmental toxicity) > 30 mg/kg/day. [2 fetuses in one high dose litter with cervical ribs not considered a significant effect].

Strain: Sprague-Dawley rats. Dose levels tested: 0, 5, 15 or 30 mg/kg/day in corn oil - subcutaneous administration.

Classification: SUPPLEMENTARY. The route of administration (subcutaneous) is not recommended by the Guidelines.

Quality Assurance Statement: Not provided. Study is circa 1976.
Good Laboratory Practice Statement: Not provided. " " " "

REVIEW

Experimental Constants:

Test Chemical: Lindane ("Gamma Benzene Hexachloride, USP") specified as being from Lot No.: 36346 and described as a white granular material with an unpleasant odor.

Study deficiency: No information on the analysis of the material as technical grade or in the dosing solutions was provided.

Test System: Sprague-Dawley rats obtained from the Charles River Laboratories, Wilmington, Massachusetts. No information on their exact age on receipt was provided. They were mated (2 females/male) bred with stock males of the same strain.

Basic Experimental Design: Four groups of presumed pregnant dams were dosed with either 0, 5, 15 or 30 mg/kg of test material in corn oil (1 ml/kg) on days 6 through 15 of gestation by subcutaneous injection. On day 19 of gestation the rats were sacrificed by chloroform and the fetuses were removed by cesarian section.

Statistics:

Statistical test	Parameter Investigated
F-test Student's t-test (with Cochran's approximation)	Maternal body weight gains Mean fetal weight Mean fetal lengths
Chi-square method	Reproduction indices

Results

A. Maternal Toxicity.

1. Survival and clinical reactions. Two high dose group rats died. There were no other deaths reported. The signs of toxicity reported in the high dose group were tremors, convulsions, urine stains, excitability and anorexia but these were reported only in a single animal. Since individual animal data were not presented, it could not be determined if the signs of tremors and convulsions were in the animal that died.

2. Food consumption and body weight. Refer to Figure 2 "Maternal Body Weights and Body Weight Changes" photocopied from the study report (attached). The mean body weight change for days 6-15 indicated a compound related effect. For example, the body weight gains for this interval were 50.3, 42.9, 38.4 ($p < 0.05$) and 11.4 ($p < 0.05$) gms. The low dose group was lower but was not significantly lower. Only the high dose group day 0 to day 19 body gain (86.4 gms, $p < 0.05$) was significantly lower

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than the control (125.3 gms). Only the food consumption for the high dose group reached a statistically significant decrease (refer to Table 1 below.

Table 1. Food consumption data for dams dosed subcutaneously with lindane.

Interval	Control	Low	Mid	High
0-6	129.9 ± 14.5	130.6 ± 12.6	133.1 ± 12.8	125.9 ± 15.1
6-11	115.2 ± 13.3	111.7 ± 10.4	103.3 ± 21.1	**54.6 ± 17.8**
11-15	93.8 ± 12.2	92.3 ± 10.0	90.4 ± 9.2	99.5 ± 16.1
15-19	107.4 ± 15.5	105.7 ± 9.8	109.7 ± 9.7	103.1 ± 14.8

3. Necropsy and organ weight data. No data on the condition of the dams for other than uterine contents was provided.

B. Uterine Data. Refer to Figure 3 "Summary of Ovarian, Uterine, and Litter Data" photocopied form the study report attached.

The study report asserts that there were no effects of the test material on the uterine parameters. There were 17, 16, 18 and 17 dams that were pregnant in each dose group. Corpora lutea and implantation site data indicated good study efficiency. The incidence of resorptions (as %) was highest in the high dose group (10.1%) whereas it was only 5.5% in the control group. Correspondingly the incidence of fetal viability was slightly lower in the high dose group (89.9%) than in the control (94.5%) the other groups were similar to the control.

Mean fetal body weight in the mid dose group was slightly higher (7.6%, $p < 0.05$) but the high dose group was lower (2%, not significant). Fetal crown rump length was similar in all dosed groups.

C. Fetal Data

Approximately 1/3 of the fetuses were prepared in Bouin's solution and evaluated for visceral effects using Wilson's technique. Whole body transverse sections of the head, thoracic and abdominal regions were reportedly taken and examined. The remaining 2/3 of the fetuses from each litter were reportedly eviscerated "examined internally" and placed in acetone. The were reportedly macerated and stained with 1% potassium hydroxide and alizarin red S and cleared with benzyl-ethyl-glycerol solution and then evaluated for degree of

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ossification and animalities.

1. Visceral Effects. There were 61, 53, 67 and 57 fetuses reportedly examined for visceral effects for the control to high dose groups respectively. Two abnormalities were reported and both were in the control group.

2. Skeletal Effects. There were 146, 119, 157, and 121 fetuses reportedly examined for the control to high dose groups respectively for skeletal effects. A total of 8 malformations were reported in the skeletal examination. Of these only "ribs-cervical" were reported only in the high dose group and 2 fetuses were affected which were from the same dam. The study report asserts that there was no evidence of "teratogenic effect" due to lindane treatment.

CONCLUSION. SUPPLEMENTARY. The study has been identified to have the following deficiencies:

- -the route of administration (subcutaneous) is not recommended in the guidelines.
- -purity of the test material not provided
- -less than 20 (16-18) dams had viable litters
- -most of data in summary tables only and little individual animal data presented
- -no analysis of the dosing solution of stability and accuracy of the dose level

TB-I does not consider that these deficiencies will alter the conclusions of the study. The route of administration, however, precludes upgrading to CORE MINIMUM for this study itself. The data, however, is considered useful in combination with other developmental toxicity studies in rats. The study supports the following "one liner":

NOEL and LEL (maternal toxicity) = 5 and 15 mg/kg/day. Decreased body weight. At 30 mg/kg/day: tremors, convulsions and death (2).

NOEL (developmental toxicity) > 30 mg/kg/day. [2 fetuses in one high dose litter with cervical ribs recognized but not considered a significant effect].

Strain: Sprague-Dawley rats. Dose levels tested: 0, 5, 15 or 30 mg/kg/day in corn oil - subcutaneous administration.

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Lindane
Haskell # 405-104, July 13, 1976
MRID # 00126269

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. V — Technical form of the active ingredient tested. (purity not provided)
2. NO — At least 20 litters/dose group for mice, rats or hamsters are available. At least 12 litters /dose group for rabbits are available (three test groups and control group).
3. V — At the high dose, maternal effects are reported as significant (or a limit dose is given, 1,000 mg/kg).
4. • V — At the low dose, no developmental toxicity is reported.
5. V — Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6. • ND — Analysis for test material stability, homogeneity and concentration in dosing medium
7. ND — Individual daily observations. Not tabulated but summarized
8. V — Individual body weights.
9. ~~NO~~ V — Individual food consumption.
10. V — Necropsy on all animals
11. V — Individual uterine examination including number of fetal deaths, early and late resorptions and numbers of viable fetuses per sex.
12. V — All ovaries examined to determine number of corpora lutea.
13. V/NO — Individual litter weights and/or individual fetal weights per sex/litter. No individual fetal weights
14. V* — Individual fetus external examination.
15. V* — Individual fetus skeletal examination for 1/3 to 1/2 of each litter for rodents and all for all rabbits.
16. V* — Individual fetus soft tissue examination.

2. 17, 16, 18 and 17 for the control to high dose groups respectively.

* 14, 15 and 16. Individual animal data not presented but summary tables presented.

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

Reviewed by: John Doherty
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Secondary reviewer: Marion Copley, DVM
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John Doherty 6/11/93
Marion Copley 6/11/93

DATA EVALUATION REPORT

STUDY TYPE: 83-3. Developmental Toxicity - rats

MRID NO.: 428080-01

TOX. CHEM. NO.: 527
PC No.: 009001

TEST MATERIAL: Technical lindane from Batch No.: 6801/403. Synonym: gamma hexachloro cyclohexane, gamma benzene hexachloride.

STUDY NUMBER(S): 4307/71/463

SPONSOR: E. Merck

TESTING FACILITY: Huntingdon Research Centre, England

TITLE OF REPORT: "Effect of Lindane on Pregnancy of the Rat"

AUTHOR(S): Anthony. K. Palmer and Michael R. Lovell

REPORT ISSUED: December 3, 1971

STUDY DATES: Not specified.

CONCLUSIONS:

NOEL and LEL (maternal toxicity): 5 and 10 mg/kg/day. Decrease body weight and food consumption. Deaths (2 dams) at 20 mg/kg/day.

NOEL and LEL (developmental toxicity): 10 and 20 mg/kg/day. Increase in pups and litters with 14th ribs and total pups affected with skeletal variations. A trend for increase in this condition at the lower levels is recognized.

Strain: CFY (derived from Charles River CD). Dose levels tested: 0, 5, 10 or 20 mg/kg/day in 0.5% carboxymethylcellulose.

Classification: CORE-MINIMUM

Quality Assurance Statement: Not provided study is circa 1971.
Good Laboratory Practice Statement: Not provided study is circa 1971.

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REVIEW

Experimental Constants:

Test Chemical: Lindane described only as from batch No.: 6801/403 and as being gamma-hexachlorocyclohexane.

Study deficiency: No information on analytical testing for purity was provided for either the technical grade product or for the product as dissolved in the vehicle (0.5% carboxymethyl-cellulose).

Test System: Specific pathogen free rats of the CFY strain obtained from the Carworth Europe Ltd. Company, Huntingdon, England. No information on their age was provided.

Basic Experimental Design: Four groups of 20 assumed pregnant rats were dosed as either control (vehicle only), or with 5, 10 or 20 mg/kg of lindane on days 6 through 15 of pregnancy. The dosing volume was 10 ml/kg. On day 20 of assumed pregnancy the rats were sacrificed by carbon dioxide euthanasia and both the dams and their uterine content were examined for effects.

Statistics: No separate section on statistical methods used was presented in the methods section. Based on inspection of the data tables the Wilcoxon test was used for assessment of skeletal variations. No other evidence of statistical comparisons was presented.

Results

A. Maternal Toxicity.

1. Survival and clinical reactions.

Two rats in the high dose group died but no deaths in the other groups were noted. Their carcasses were autolyzed and their cause of death in relation to lindane toxicity was not reported. No clinical reactions to treatment were reported. The deaths, however, are considered to be related to lindane administration.

2. Body weight and food consumption.

The dams were weighted on days 1, 3, 6, 10, 14, 17 and 20 of pregnancy. The study asserts that body weight decreases were noted in the groups dosed with 10 and 20 mg/kg/day. There was a similar decrease in food consumption. Figure 2 ("Group mean bodyweight change of dams with viable young" photocopied from the study report, attached) and Tables 1 ("Group mean food consumption") and 2 ("Group mean body weights of dams with viable young", both photocopied from the study report) illustrate the effect on body weight and/or food consumption.

CONCLUSION (body weight): NOEL and LEL: 5 and 10 mg/kg/day. Decreased body weight gain at 10 and 20 mg/kg/day.

B. Uterine Data. Refer to Table 4 :Group mean litter data" photocopied from the study report attached.

There were 17, 16, 18 and 15 dams for the control to high dose groups that had viable young. This is less than the 20 for each dose level recommended by the current guidelines but the starting number of dams was too low to expect to result in the recommended number of dams with viable fetuses. Corpora lutea and implantation data all indicated no in-efficiencies in the pregnancy of the dams. One rat in each of the low and mid dose groups had total resorptions. The mean number of resorptions, however, was 0.8 for the control and 0.7 for all other groups, indicating there was no compound related effect on this parameter. The mean number of viable young was highest in the high dose group (12.3 vs. 11.0 in the control group). Mean litter weight was also highest in the high dose group and a trend toward increased mean fetal weight was apparent (41.26, 42.20, 43.30 and 45.12 gms for the control to the high dose group respectively). Mean pup weight was, however, slightly lower for the high dose group (3.69 gm) than the control (3.73 gm). Thus, there were no effects on the course of pregnancy due to lindane.

C. Necropsy and organ weight data.

Gross pathology and organ weight data were reported. The organs weighed were: brain, pituitary, heart, lungs, liver, spleen thymus, kidneys, thyroid, adrenals and ovaries. No compound related effects observable at gross necropsy or on organ weights were evident.

D. Fetal Data

The study report asserts that all pups were examined externally on removal from the uterus. One third of the pups were preserved in Bouin's solution for subsequent free hand sectioning (Wilson's technique) to assess for visceral abnormalities. The remaining 2/3 were preserved in alcohol for subsequent dissection and skeletal examination. Only summary tables of the visceral and skeletal effects were presented.

1. Visceral Effects. There were 51, 43, 49, and 53 fetuses reported as being examined for the control and high dose groups respectively. Of these, only a single lesion ("increased renal pelvic cavitation") was noted in the low dose group. No other groups were reported as having visceral abnormalities.

2. Skeletal Effects. There were 135, 133, 153 and 131 pups reported examined for skeletal effects. Table 6 ("Incidence of

skeletal effects", photocopied from the study report, attached) illustrates the results of the skeletal assessment.

The study report asserts that the data indicate that there is a compound related increase in the incidence of pups with extra (14th) ribs and in the total incidence of variant pups in the high dose group based on group mean litter percent. Table 1 below illustrates the effects data on 14th ribs.

Table 1. Pup and litter data for 14th ribs and total pups affected with skeletal variants in rat lindane developmental toxicity study.

Dose Level	With 14th ribs		Total Pups affected	
	Pups[1]	Litters(%)	Pups	Litters(%)
Control	17/135 (13%)	12.7	58/135 (43%)	43.4
5 mg/kg	29/133 (22%)	21.0	70/133 (53%)	52.7
10 mg/kg	49/153 (32%)	31.7	92/153 (60%)	59.5
20 mg/kg	54/131 (31%)	40.6*	89/131 (68%)	68.0**

* $p < 0.05$ and ** $p < 0.01$, study report statistics (Wilcoxon test).
1. Data are number of pups with condition/number of pups examined and the percentage affected is in ().

Table 1 above shows that there is a pronounced trend indicating a possible treatment related effect starting at the lowest test dose level. The data, however, reach statistical significance for the high dose test group only. Since this is a rather common variant, TB-I has opted to agree with the study report and conclude that the data indicate a treatment related response at the high dose test group only. The presence of the trend at lower dose levels is recognized.

The report also included some historical control data (ranges only) for the Charles River CD rat (from which the CFY rat is derived) which indicate that the range for litters with extra ribs is 8-41% and for litters with total variants is 35-68%. Thus, the response in this study for extra ribs (40.6%) and total variants (68%) is within the upper limit of the historical control for the high dose group. The increase, however, is still considered to be treatment related for the high dose group with the presence of a trend at lower doses.

CONCLUSION. This study is CORE-MINIMUM. The results of the study with subcutaneous administration (Hazleton Study No.: 405-104, July 13, 1976) support the conclusion for classification as MINIMUM. The following deficiencies in the conduction or reporting of the study were noted.

-percentage purity of the test material

-less than 20 dams with viable litters (there were 15-18)
-no analysis of the test material in the dosing solution for stability and accuracy of the dosing
-no individual food consumption data
-much of data is in summary tables only and not accompanied by individual animal data

These deficiencies, however, do not warrant that the study be repeated. The data support the following "one-liner":

NOEL/LEL (maternal toxicity): 5/10 mg/kg/day. Decrease body weight and food consumption.

NOEL/LEL (developmental toxicity): 10/20 mg/kg/day. Increase in pups and litters with 14th ribs. A trend for increase in this condition at the lower levels is recognized.

Strain: CFY (derived from Charles River CD). Dose levels tested: 0, 5, 10 and 20 mg/kg/day in 0.5% carboxymethylcellulose.

Lindane
Huntingdon # 4307/171/463
MRID [redacted]-428086-01 December 3, 1971

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. ✓ Technical form of the active ingredient tested. (purity not stated)
2. NO At least 20 litters/dose group for mice, rats or hamsters are available. At least 12 litters /dose group for rabbits are available (three test groups and control group).
3. ✓ At the high dose, maternal effects are reported as significant (or a limit dose is given, 1,000 mg/kg).
4. • ✓ At the low dose, no developmental toxicity is reported.
5. ✓ Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6. • NO Analysis for test material stability, homogeneity and concentration in dosing medium
7. — Individual daily observations. summarized
8. ✓ Individual body weights.
9. NO Individual food consumption.
10. ✓ Necropsy on all animals
11. ✓ Individual uterine examination including number of fetal deaths, early and late resorptions and numbers of viable fetuses per sex. Summary table
12. ✓ All ovaries examined to determine number of corpora lutea.
13. XX Individual litter weights and/or individual fetal weights per sex/litter.
14. XX Individual fetus external examination.
15. XX Individual fetus skeletal examination for 1/3 to 1/2 of each litter for rodents and all for all rabbits.
16. XX Individual fetus soft tissue examination.

2. 17, 16, 18 and 15 for the control to high dose respectively.

XX 13, 14, 15 and 16 data in summary tables.

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

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Secondary reviewer: Marion Copley, DVM
Section IV, Toxicology Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 83-3. Developmental toxicity rabbits (subcutaneous)

MRID NO.: 00062658 (~~Acc. No.~~) TOX. CHEM. NO.: 527
PC No.: 009001

TEST MATERIAL: Lindane (lot or batch not stated).

STUDY NUMBER(S): 405-103

SPONSOR: Reed and Carnick Research Institute

TESTING FACILITY: Hazleton Laboratories America

TITLE OF REPORT: "Teratology Study in Rabbits Lindane (Gamma benzene hexachloride, U.S.P.)"

AUTHOR(S): Frederick E. Reno, Ph.D., DIrector of Toxicology

REPORT ISSUED: August 6, 1976

STUDY DATES: Not provided.

CONCLUSIONS:

Maternal Toxicity NOEL and LEL = 5 and 15 mg/kg/day. Body weight and food consumption decrease, clinical reaction and death (1). 45/30 mg/kg/day: severe toxicity (death).

Developmental toxicity: NOEL > 15 mg/kg/day.

New Zealand White rabbits. Dose levels tested O, 5, 15 or 45/30 dose level terminated because of excessive toxicity) mg/kg/day subcutaneously administration in corn oil.

Classification: SUPPLEMENTARY. Non guideline route of exposure (sub-cutaneous) and high mortality at high dose. Study not upgradeable by itself.

Quality Assurance Statement: Not provided (study is circa 1976)
Good Laboratory Practice Statement: " " " " " "

REVIEW

Experimental Constants:

Test Chemical: Lindane ("gamma benzene hexachloride") provided by the U.S.P. and described as a white granular material with an unpleasant odor. No other information was provided. The test material was dissolved in corn oil prior to administration.

Test System: New Zealand White rabbits obtained from the Bunnyville Farms, Littletown, Pennsylvania. Following stimulation of ovulation by the intravenous administration of 250 IU of human chorionic gonadotrophin, the does were artificially impregnated with sperm from sires the Hazleton stocks.

Basic Experimental Design: Four groups of 15 does were assigned to groups which were dosed as 0, 5, 15 and 45 mg/kg/day. The high dose group was reduced to 30 mg/kg/day after day 9 of gestation due to the high toxicity of lindane. The high dose group will be referred to as the 45/30 dose group in this DER and in other references to this study. The dosing was by the subcutaneous route (0.5 ml/kg) on days 6 through 18 of gestation inclusive. On day 29 of gestation the surviving does were sacrificed by intravenous air embolism and the fetuses removed and examined for both visceral and skeletal effects.

Statistics: The following statistical assessments were utilized.

Statistical Test	Parameter
F-test (analysis of variance) Student's "t" test	Body weight gains Mean fetal weights and lengths
Cochran's approximation	When variances differed significantly
chi-square	Reproduction indices

Results

A. Maternal Toxicity.

1. Mortality and clinical signs. One mid dose and 14 of the 15 high dose does died between days 10 and 26 of gestation. The experiment at 45/30 mg/kg/day was discontinued because of the high toxicity of lindane and their fetuses were not examined.

In the mid dose group, decreased activity and immobilized rear quarters were the principle signs noted. The low dose group was not reported as displaying any signs.

2. Body weight and food consumption. Body weight gain for the low dose group was essentially equivalent (but possibly higher) to the controls. The mid dose group was markedly affected with a

weight loss (126.7 gms) during the interval of days 6 - 20 of gestation. During this period the control group gained 218 gms. Food consumption was reported to be slightly higher in the low dose group but markedly lower in the mid and high dose groups.

3. Gross pathology. Note: Poor photocopying obscured the test and tables for this section. It could be seen that the does in the mid and high dose group had indications of differences in the texture of the liver.

NOEL and LEL (maternal toxicity): 5 and 15 mg/kg/day. Body weight and clinical reactions including death. At 45/30 mg/kg/day: severe toxicity (death).

B. Uterine Data. Refer to "Figure 3 - Summary of Ovarian, Uterine, and Litter Data" photocopied from the study report attached. [The high dose group (45/30 mg/kg/day) is not discussed]. There were 11, 11 and 15 pregnant does for the control, 5 and 15 mg/kg/day dose groups. One dam in the 15 mg/kg/day group aborted and died. There were no differences related to resorption or dead fetuses indicative of toxicity to lindane. If anything, the incidence of dead fetuses was highest in the control group.

C. Fetal Data. There were 49, 54 and 63 live fetuses that had mean weights of 44.27, 47.90 and 44.68 gms for the control and 5 and 15 mg/kg/day dose groups. The mean crown rump lengths for these groups were 9.06, 9.04 and 8.85 respectively. The slightly lower figure for the 15 mg/kg//day group is not considered biologically significant.

1. Visceral Effects. This examination was done by Wilson's freehand section for 25, 25 and 29 fetuses from the three surviving groups. Only one pup was reported to have a defect. A mid dose group pup and a dilated renal pelvis.

2. Skeletal Effects. @5, 29 and 34 fetuses were examined for skeletal defects. No compound related increases in skeletal effects were reported.

CONCLUSION. This study is SUPPLEMENTARY. The subcutaneous route of administration is not a preferable method of compound administration. There was a high rate of deaths in the high dose group rendering this group unacceptable. Individual animal data were not provided for litter weight, external and skeletal examinations. No analysis of the test material for stability, homogeneity and concentration in the dosing solutions. The study itself is not considered upgradeable but the data may be used in conjunction with another oral developmental toxicity study with lindane in rabbits to help meet the series 83-3 guideline

requirement.

The following "one liner" is supported.

Maternal Toxicity NOEL/LEL = 5/15 mg/kg/day. Body weight and food consumption decrease, clinical reaction and death (1). 45/30 mg/kg/day: severe toxicity (death).

Developmental toxicity: NOEL > 15 mg/kg/day.

New Zealand White rabbits. Dose levels tested 0, 5, 15 or 45/30 dose level terminated because of excessive toxicity) mg/kg/day subcutaneously administration in corn oil.

Lindane
Huntingdon # 430S/71/469
December 2, 1971
MRID NO. 428080-02

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. ✓ Technical form of the active ingredient tested.
2. NO At least 20 litters/dose group for mice, rats or hamsters are available. At least 12 litters /dose group for rabbits are available (three test groups and control group).
3. NO At the high dose, maternal effects are reported as significant (or a limit dose is given, 1,000 mg/kg).
4.* ✓ At the low dose, no developmental toxicity is reported.
5. ✓ Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6.* NO Analysis for test material stability, homogeneity and concentration in dosing medium
7. S Individual daily observations.
8. ✓ Individual body weights.
9. NO Individual food consumption.
10. ✓ Necropsy on all animals
11. ✓ Individual uterine examination including number of fetal deaths, early and late resorptions and numbers of viable fetuses per sex.
12. ✓ All ovaries examined to determine number of corpora lutea.
13. S Individual litter weights and/or individual fetal weights per sex/litter.
14. S Individual fetus external examination.
15. S Individual fetus skeletal examination for 1/3 to 1/2 of each litter for rodents and all for all rabbits.
16. S Individual fetus soft tissue examination.

2. There were 13, 12, 11 and 10 does in 4 litters.

5 = summary table only.

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