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

009851

NOV 27 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Endosulfan - Re-Evaluation of Rat and Rabbit Developmental Toxicity and Rat 2-Generation Reproduction Studies; Reconsideration of the RfD.

TO: Lois Rossi
Product Manager (74)
Reregistration Branch, SRRD (H7508W)

FROM: Linda L. Taylor, Ph.D. *Linda Taylor* 11/2/92
Toxicology Branch II, Section II
Health Effects Division (H7509C)

Thru: K. Clark Swentzel. *K. Clark Swentzel* 11/2/92
Toxicology Branch II, Head Section II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. *mvGemert* 11/3/92
Chief, Health Effects Division (H7509C)

Registrant: Hoechst Celanese Corporation
Chemical: 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
Synonyms: Endosulfan; Thiodan; Benzoepin; Endocide
Shaughnessy No.: 079401
CASWELL No.: 420
MRID No.: 00055544, 00094837, and 148264/460002-031 (Accession #256127)

Comment: The HED RfD/Peer Review Committee met on several occasions regarding Endosulfan and requested reevaluation of both the rat and rabbit developmental toxicity studies and the 2-generation reproduction study. These three studies were re-reviewed and presented to the Committee. The re-reviews of both the rabbit developmental toxicity study (DER dated 12/24/91) and the rat 2-generation reproduction study (DER dated 6/23/92; Document # 009552) concurred with the original reviews, in that the classification of each remains Core Minimum. The re-review of the rat developmental toxicity study (DER dated 12/24/91) concluded that the study was not adequate and was reclassified Core

Supplementary (not upgradeable). In a meeting on March 20, 1992, the Committee concluded that the rabbit study was adequate and recommended that the rat developmental toxicity study be repeated. In a meeting on October 1, 1992, the Committee concluded that the reproduction study was adequate but recommended that the chronic rat and dog studies be used as co-critical studies for setting the RfD.

The purpose of this memo is to inform you of the status (classification) of these three studies and transmit a copy of each new DER. Additionally, a copy of the RfD Committee's memo regarding the new RfD for Endosulfan is attached.

cc: Karen Samek/PM 50, SRRD (H7508W)
George Larocca/PM 15, RD (H7505C)



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

L. Taylor

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10/13/92

MEMORANDUM:

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: RfD/Peer Review Report of Endosulfan
CAS No. 115-29-7
EPA Chem. No. 079401
Caswell File No. 420
Reg. Group: List A (6A)

FROM: George Z. Ghali, PhD
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

G. Ghali 10.5.92

TO: George Larocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (H7505C)
and
Lois Rossi, Chief
Reregistration Branch
Reregistration and Special Review Division (H7508)

The Health Effects Division RfD/Peer Review Committee met on October 1, 1992 to consider issues related to the reproductive toxicity of endosulfan and their impact on the current RfD for this chemical.

Endosulfan had been the subject of several meetings by the HED RfD/PR Committee. On March 20, 1992, the discoloration of the kidney tubules observed in the reproduction study at all dose levels (and in other studies with endosulfan) had been dismissed and was not considered of toxicological significance. The lowest dose level in this study was considered to be a "no-observable effect level", and the middle dose level was considered an LEL for organ weight changes. Other effects observed at the middle dose levels included significant decrease in mean litter size.

Although the Committee questioned effects seen at the middle dose level, it was recommended in the meeting of March 20, 1992 that an RfD be established, at least tentatively, on the basis of the "no-observable effect level" of 3 ppm (0.15 mg/kg/day) demonstrated in this rat reproduction study, using an uncertainty factor (UF) of 100 to account for the interspecies extrapolation and intraspecies variability, and at the same time recommended the reevaluation of the study. Since the effects seen at the middle dose level were questionable, this RfD was considered to be a provisional value until the reevaluation of the study.

In the meeting of October 1, 1992 the Committee dismissed effects seen at the mid-dose level in the reproduction study. Although the Committee agreed with most of the reviewer's interpretation of the data, the Committee concluded that compound-related toxicity was not evident at the mid-dose level. Although the changes in the heart and liver weights reported in the mid-dose of the F0 adult males and females respectively were statistically significant, the Committee noted that these effects were slight and limited to one sex of one litter and occurred only in one generation. In view of this and in the absence of histopathological changes in the liver, the Committee concluded that these statistically significant changes in organ weights should not be considered of biological significance. The NOEL for systemic toxicity was therefore considered to be the middle dose level (15 ppm or 0.75 mg/kg/day) based upon decreased body weight gain observed at the high dose level (70 ppm or 3.5 mg/kg/day). No reproductive toxicity was reported at any dose level. The NOEL for reproductive toxicity was considered to be 75 ppm or 3.5 mg/kg/day.

It was recommended that an RfD be established for endosulfan on the basis of a "no-observable effect level" of 0.6 mg/kg/day for reduced body weight gain in males and females, and increased incidence of marked progressive glomerulonephrosis and blood vessel aneurysms in males observed at 2.9 and 3.8 mg/kg/day for males and females respectively demonstrated in a long-term feeding study in the rat. An uncertainty factor of 100 was recommended to account for interspecies extrapolation and intraspecies variability. The long-term feeding study in dogs demonstrating a no-observable level of 0.6 mg/kg/day (based on actual food intake) was considered to be supportive and co-critical.

A. Individuals in Attendance

1. Peer Review Committee and Associates Present in One or Both Meetings (signature indicates concurrence with the peer review unless otherwise stated).

Reto Engler

Karl Baetcke

Marcia Van Gemert

Henry Spencer

Gary Burin

William Sette

George Ghali

Rick Whiting

Roger Gardner

Chris Taylor

Carl D. Baetcke

Marcia van Gemert

Henry Spencer

Gary Burin

William Sette

G. Ghali

Rick Whiting

Roger Gardner

2. Peer Review Members and Associates in Absentia (committee members and associates who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the committee).

William Burnam

William Burnam

3. Scientific Reviewer (committee or non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report).

Linda Taylor

Clark Swentzel

Linda Taylor

Clark Swentzel

- CC. P. Fenner/Crisp
R. Schmitt
K. Dearfield
M. Copley
L. Hansen
E. Saito
J. Kariya

B. Conclusions and Recommendations

Although the Committee agreed with most of the reviewer's interpretation of the reproduction study, the Committee felt that compound-related toxicity was not evident at the mid-dose level. Although the changes in the heart and liver weights reported in the mid-dose of the F0 adult males and females respectively were statistically significant, the Committee noted that these effects were slight and limited to one sex of one litter and occurred only in one generation. In view of this and in the absence of histopathological changes in the liver, the Committee concluded that these statistically significant changes in organ weights should not be considered of biological significance. The NOEL for systemic toxicity was therefore considered to be the middle dose level (15 ppm or 0.75 mg/kg/day) based upon decreased body weight gain observed at the high dose level (70 ppm or 3.5 mg/kg/day). No reproductive toxicity was reported at any dose level. The NOEL for reproductive toxicity was considered to be 75 ppm or 3.5 mg/kg/day.

C. Reference Dose (RfD)

The Committee recommended that an RfD be established for endosulfan on the basis of a "no-observable effect level" of 0.6 mg/kg/day for reduced body weight gain in males and females, and increased incidence of marked progressive glomerulonephrosis and blood vessel aneurysms in males observed at 2.9 and 3.8 mg/kg/day for males and females respectively demonstrated in a long-term feeding study in the rat. An uncertainty factor of 100 was recommended to account for interspecies extrapolation and intraspecies variability. The long-term feeding study in dogs, demonstrating a no-observable level of 0.6 mg/kg/day (based on actual food intake), was considered to be supportive and co-critical.

DEC 24 1991

009851

GUIDELINE: 83-3

Primary Review by: Gary J. Burin, Ph.D., D.A.B.T.
SACB/HED

G. J. Burin

Secondary Review by: Albin Kocialski, Ph.D.
Section Head, SACB /HED

AK

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3

EPA Identification No.s: EPA MRID (Accession) No. 243707
Caswell No. 420

Test Material: FMC 5462

Synonyms: Endosulfan

Sponsor: FMC

Study Number(s): Raltech Study No. 79041

Testing Facility: Raltech Scientific Services, Madison, Wisconsin

Title of Report: Teratology Study with FMC 5462 in Rats

Author(s): W.P. Fung

Report Issued: October 2, 1980

Conclusions: An NOEL was not established for developmental toxicity (<0.66 mg/kg) based on an increased incidence of misaligned vertebrae at all dose levels. The NOEL for maternal toxicity is 0.66 mg/kg.

Core Classification: Supplementary data

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: Purity: 97.3%
Description: technical grade
Lot No.: 3/827

Vehicle(s): Corn oil

Test Animal(s): Species: Rat
Strain: CD Sprague Dawley
Source: Charles River, Portage, Michigan
Age: 8 weeks
Weight: Mean weights of animals assigned to each group ranged from 264 to 273g. SD ranged from 17 to 20g.

B. Study Design

This study was designed to assess the developmental toxicity potential of endosulfan when administered by gavage to pregnant rats on gestation days 6 through 19, inclusive.

Mating

Females were naturally mated by housing each virgin female with one male. Females were checked daily for the presence of a vaginal plug or sperm in the vaginal smear.

Table 1. Experimental design

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	30
Low Dose	0.66	25
Mid Dose	2	25
High Dose	6	35

Dosing:

All doses were in a volume of 5 ml/kg of body weight/day prepared every 4 to 6 days during the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was based on gestation day 6 body weight.

Observations

The animals were checked for mortality or abnormal condition on days 0, 6, 9, 12, 15, 18 and 20. Dams were

sacrificed on day 20 of gestation. Dams were weighed on gestation days 0, 6, 9, 12, 15, 18 and 20. Ovaries and uterus of the dams were examined at sacrifice and fetuses were weighed and measured. All fetuses were examined grossly and cleared for skeletal staining. Prior to clearing the skulls from one half of the fetuses were examined by free hand sectioning and the other half examined after staining.

Statistical analysis

The following statistical analysis methods were employed: The body weights of dams were analyzed by analysis of variance and significant treatment means were compared using Dunnett's multiple comparison test. Kruskal-Wallis testing was conducted on litter data and contingency table analysis was used to compare the numbers of litters with abnormalities between treatment groups. Percentage of litters with abnormalities were compared using the Kruskal-Wallis test.

Compliance

A claim of confidentiality was made. A signed Statement of compliance with EPA GLP's was provided.

C. Results

1. Maternal Toxicity

Mortality

Seven high dose dams died during the course of the study and 5 of these deaths appeared to be the result of improper gavage. One control animal died on test.

Clinical Observations

Toxic signs in the high dose included face rubbing, brown exudate, rough coat, flaccidity and hyperactivity. Occasional face rubbing was also observed in the mid dose group.

Body Weight

The mean body weight gains are shown below:

Table I: Body Weight Gains (grams)^a

Group:	Dosing Period	Entire Gestation Period	Corrected Body Weight Gains ¹
Control	128	160	75
LDT	124	155	70
MDT	117	151	64
HDT	79	108*	30*

1 = corrected body weight gain for entire gestation period = body weight gain for entire gestation period minus gravid uterus weight.

a = Data extracted from study report Table 3

* = $p < .05$

Body weight gains during the dosing period, throughout the entire gestation period and corrected for gravid uterine weight were significantly less in the high dose group than in controls. The latter was also significantly ($p < 0.05$) less than control for the mid dose group.

Food Consumption

Food consumption was not measured. Visual inspection revealed no alterations in food intake.

Gross Pathological Observations

No gross pathological observations appeared to be related to treatment.

Cesarean section Observations

Table 2: Cesarean Section observations^a

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	30	25	25	35
#Animals Examined	29	25	25	28
Pregnancy Rate (%)	97	92	100	96.4
Maternal Wastage				
#Died	1	0	0	7
#Died/pregnant	1/28	0/23	0/25	7/27
#Non pregnant	1	2	0	1
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Corpora Lutea/dam	16	16	17	16
Implantations/Dam	15	14	16	15
Live Fetuses/Dam	15	14	14	15
Resorptions/Dam	0.4	0.5	1.4	0.3
Total Dead Fetuses	0	0	0	0
Mean Fetal Weight (gm)	3.8	4.0	3.9	3.5

Preimplantation Loss(%)	6.4	8.4	5.1	5.9
Postimplantation Loss(%)	2.6	3.5	8.8	2.0
Sex Ratio (% Male)	50.5	48.1	47.9	45.8

^a = Data extracted from study report Table 5

Mean fetal weight and fetal length (not shown) was significantly decreased in the high dose group. Other parameters did not appear to be effected by treatment.

2. Developmental Toxicity

Out of a total of 27 litters containing 405 live fetuses at the high dose level, one fetus had skin of the upper forelimb webbed to the chest and 5 other animals of this litter had edema and lordosis, a fetus from another litter was found to have edema and short limbs, two litters each had a fetus with clubbed hind left limbs and two litters had fetuses with cardiovascular abnormalities. In the control group, one fetus was found to have a small tail and anus and one fetus had a poorly developed nasal concha. Microstomia was observed in one fetus in the 0.66 mg/kg group. Table 3 presents selected skeletal observations.

Table 3. Skeletal Examinations

<u>Observations</u>	<u>Control</u>	<u>Low Dose</u>	<u>Mid Dose</u>	<u>High Dose</u>
#pups(litters) examined	327(22)	309(22)	348(24)	389(24) ⁶
Sternebrae				
misaligned	3(1)	13(10)	12(8)	9(7)
poorly ossified	60(12)	68(14)	31(11)	126(22)
unossified	48(10)	57(12)	20(11)	153(23)
Extra ribs	1(1)	0(0)	1(1)	4(4)

D. Discussion/Conclusions

a. Maternal Toxicity: Pronounced maternal toxicity in the forms of clinical indications and decreased body weight gain were observed at the high dose level. Decreased corrected body weight gain and less frequent clinical signs of toxicity (face rubbing) were observed at the mid dose level (2 mg/kg). The NOEL for maternal toxicity was therefore 0.66 mg/kg.

b. Developmental Toxicity:

- i. Deaths/Resorptions: No effect under conditions of test.
- ii. Altered Growth: Decreased body weight and length at the high dose.
- iii. Developmental Anomalies: An increase in misaligned sternbrae was observed in all treated groups compared to concurrent controls. The increase in litters and fetuses affected at each dose level was above that reported in the historical data base (18% of litters and 1.85% of fetuses based upon the examination of 65 litters and 863 fetuses). However, the variability between studies was not reported. An increased incidence of litters with extra ribs, poorly ossified and unossified sternbrae was observed at the high dose level. More detailed historical control data may be useful in determining whether the apparent increase in misaligned sternbrae is due to an unusually low incidence in the concurrent control group and within the variability observed between studies. In the absence of such information, it is recommended that this finding be considered to be compound-related.
- iv. Malformations: No apparent effect under the conditions of the test.

D. Study Deficiencies: A NOEL for developmental toxicity was not established based upon an increased incidence of misaligned sternbrae at each dose level. The historical control data was not reported on a study by study basis. Food consumption was not measured.

E. Core Classification: Core Supplementary Data.

Maternal NOEL = 0.6 mg/kg
Maternal LOEL = 2 mg/kg
Developmental Toxicity NOEL = <0.6 mg/kg
Developmental Toxicity LOEL = 0.6 mg/kg

Endosulfan

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Page is not included in this copy.

Pages 13 through 18 are not included.

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

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REGULATORY AND COMPLIANCE DIVISION

MEMORANDUM

SUBJECT: Evaluation of Rat Teratology Study of Endosulfan (Thiodan)
CAS# 420 Accession# 243707

FROM: Gary J. Burin, Toxicologist
Toxicology Branch, HED (TS-769)

Gary J. Burin 4/20/81

TO: G. LaRocca (15)
Registration Division (TS-757)

THRU: Chris Chaisson, Acting Chief
Toxicology Branch, HED (TS-769)

Submitted By: FMC Corp.
Agricultural Chemical Group
2000 Market Street
Philadelphia, Pa. 19103

Review of Data:

Teratology Study of Endosulfan (Thiodan), Rats. Performed by Raltech Scientific Services and submitted by FMC Corp., November 11, 1980.

Virgin female CD Sprague Dawley rats (8 weeks old) were treated with 0, 0.66, 2.0 or 6.0 mg/kg of FMC 5462 (Endosulfan, 97.37% pure, Lot No. 3/827) in corn oil after being mated with virgin males of the same strain. Treatment, by daily oral gavage, occurred on days 6-19 of gestation with test material administered in 5 ml/kg of corn oil. Although the original protocol specified 25 animals per treatment group, ten additional animals were added to the high dose group (due to mortality among the original animals) and five additional animals were added to the control group (due to a loss of some tissues during processing).

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Dams were weighed on gestation days 0, 6, 9, 12, 18 and at sacrifice on day 20. Observation for abnormal signs occurred twice daily throughout the test period.

On day 20 dams were asphyxiated with CO₂. The ovaries and uterus were removed from each animal subsequent to a midline laparotomy. The ovaries were examined grossly and the number of corpora lutea were counted. The gravid uterus was also examined grossly and was weighed. Conceptuses were removed and the number and location of live and dead fetuses, resorptions, empty sites and gross abnormalities were recorded.

Fetuses were sexed, measured, weighed, examined grossly and given a visceral examination. Freehand razor sections of the heads of one-half of the fetuses was performed and all fetuses were stained and examined for skeletal abnormalities.

Dams were grossly examined for internal and external abnormalities. Apparent lesions were preserved for possible future histopathological examination.

Raw data sheets for each dam and fetus were included in the final report. Summary sheets were also submitted for a variety of parameters. Statistical comparisons of fetal gross, visceral and skeletal abnormalities were performed using the Chi Squared method. All other parameters were compared using the Kruskal-Wallis test.

Results:

Maternal toxicity was apparent in the high dose group in the form of significantly reduced body weights and body weight gain during gestation ($p < .01$). Toxic signs observed in the high dose group included face rubbing (20/35 animals), brown exudate (4/35), rough coat (5/35), flaccidity (8/35) and hyperactivity (11/35).

Seven high dose dams died during the course of the study and five of these deaths were apparently the result of the improper gavage of the animals (the gavage tube inserted into the lungs rather than the esophagus). The remaining two deaths may have been compound related as suggested by a decrease in body weight prior to death. However, these two animals were not reported to have exhibited signs of toxicity prior to death. One control animal also died on test, apparently from improper gavage.

Maternal toxicity was not demonstrated in the low or mid-dose groups. No mortality was reported in these groups and body weights and gains in body weight were not significantly affected. Face rubbing was reported in 6/25 of mid dose animals and alopecia was reported in 1/25. No face rubbing was reported in low dose animals or controls.

One animal in the control, one high dose and two low dose animals were not pregnant at necropsy. Fetal and embryolethality were not apparent in any group and all pregnant animals had viable litters. Mean fetal weight and crown-rump length were significantly reduced ($p < .05$ and $p < .01$, respectively) in the 6 mg/kg group. No effect on fetal weight or length was found in the low or mid-dose groups.

A number of external and visceral abnormalities were observed in the 6 mg/kg group. Out of a total of 27 litters containing 405 fetuses, one fetus had skin of the upper forelimb webbed to the chest and 5 other animals of this litter had edema and lordosis, a fetus from another litter was observed to have edema and short limbs (although the short limbs were not confirmed on skeletal examination), one fetus from each of two high dose litters had clubbed hind left limbs and one fetus from each of two high dose litters had cardiovascular abnormalities (a hypoplastic aortic arch was present in one fetus and the heart of another was small and displaced within the thoracic cavity). There was also a significant increase in the incidence of small 4th and unossified 5th sternbrae ($p < .05$ and $p < .01$, respectively) in fetuses from the high dose group.

The only statistically significant developmental effects noted in the low and mid-dose groups were misaligned sternbrae ($p < .05$ in each group). A single instance of microstomia was found in the low dose group.

Conclusions:

A number of skeletal, visceral and external anomalies, as well as significant reductions in size and weight, were reported in fetuses from dams administered 6.0 mg/kg on a daily basis on days 6-19. However, at this dose level maternal toxicity was evident in the form of decreased body weight and body weight gain ($p < .01$) and clinical observations indicating CNS stimulation.

At lower dose levels, no compound-related terata were apparent although misaligned sternbrae were noted. This effect can be considered a variation, rather than a teratogenic response. The NOEL for fetal and maternal toxicity in this study is therefore considered to be 2 mg/kg.

Core-Classification

Core-Minimum Data. A positive control group was not used and maternal food consumption was not quantified.

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GUIDELINE: 83-3

Primary Review by: Gary J. Burin, Ph.D., D.A.B.T. *GJB*
SACB/HED

Secondary Review by: Albin Kocialski, Ph.D. *AK*
Section Head, SACB /HED

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rabbit
Guideline: 83-3

EPA Identification No.s: EPA MRID (Accession) No. 246792
Caswell No. 420

Test Material: FMC 5462

Synonyms: Endosulfan

Sponsor: FMC

Study Number(s): Raltech Study No. 80070

Testing Facility: Raltech Scientific Services, Madison, Wisconsin

Title of Report: Teratology Study with FMC 5462 in Rabbits

Author(s): W.P. Fung

Report Issued: July 27, 1981

Conclusions: Maternal toxicity was observed at the highest dose tested (1.8 mg/kg/day). Developmental toxicity not was observed at any dose level.

Core Classification: Core minimum

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: Purity: 97.3%
Description: technical grade
Lot No.: not specified

Vehicle(s): Corn oil

Test Animal(s): Species: Rabbit
Strain: New Zealand white
Source: Hoppers Unlimited, Verona, Wisconsin
Age: "Young" (not specified)
Weight: 3275± 309 g on day 0 for control animals. Other groups had similar means and standard deviations.

B. Study Design

This study was designed to assess the developmental toxicity potential of endosulfan when administered by gavage to pregnant rabbits on gestation days 6 through 28, inclusive. Does were sacrificed on day 29G.

Mating

Females were double mated to proven bucks by housing each virgin female with one male. Females were checked daily for the presence of a vaginal plug or sperm in the vaginal smear. Females were randomly distributed across groups.

TABLE 1 Experimental design

<u>Test Group</u>	<u>Dose by gavage mg/kg/day</u>	<u>Number of females</u>
1 Cont (vehicle)	0.0	20
2 Low (LDT)	0.3	20
3 Mid (MDT)	0.7	20
4 High (HDT)	1.8	26

Dosing

All doses were in a volume of 5 ml/kg of body weight/day prepared every 4 to 6 days during the dosing-period. The dosing solutions were analyzed for concentration and stability. Dosing

was based on gestation day 6 body weight.

Observations

The animals were observed twice daily for mortality. Does were sacrificed on day 29 of gestation. Dams were weighed on gestation days 0, 6, 13, 19, 25 and at the time of sacrifice. Ovaries and uterus of the dams were examined at sacrifice and fetuses were weighed and measured. All fetuses were examined grossly and cleared for skeletal staining. Prior to clearing the skulls from one half of the fetuses were examined by free hand sectioning and the other half examined after staining.

Statistical analysis

Day 0 and uncorrected body weights were analyzed by analysis of variance and, where significant, by Dunnet's multiple comparison test. Other weights were analyzed by covariate statistics. Gross, visceral and skeletal effects were analyzed using the Chi-square statistic with percentage of litter effects analyzed by the Kruskal-Wallis test. Other litter parameters were also analyzed using the Kruskal-Wallis test.

Compliance

A claim of Confidentiality Claim was made. A signed Statement of compliance with EPA GLP's was provided.

C. Results

1. Maternal Toxicity

Mortality

Four does died during the course of the study in the high dose group. Although the cause of death was not established for these animals, oil in the lung or trachea was observed suggesting either regurgitation or improper gavage.

Clinical Observations

Toxic signs in the high dose group included convulsions, rapid breathing, salivation and hyperactivity.

Body Weight

The mean body weight gains are shown below:

TABLE 2 Body weight change

Dose level (mg/kg/day)	Av. body weight change (g/rabbit)				
	0-6G	7-12G	13-18G	19-29G	Corrected
0	186	64	111	43	5
0.3	96	128	108	24	4
0.7	150	134	98	-16	35
1.8	139	97	115	-47	-17

Body weight gains during the days 19-29 and corrected for gravid uterine weight at sacrifice were less in the high dose group than in controls. The former was also less than control for the mid dose group. However, these differences were not statistically significant.

Food Consumption

Food consumption was not measured. Visual inspection revealed no alterations in food intake.

Gross Pathological Observations

One animals which died on test showed evidence of hemorrhagic activity. No other gross pathological observations appeared to be related to treatment.

Cesarean Section Observations

Table 3: Cesarean Section observations^a

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	20	20	20	26
#Animals Examined	20	20	20	22
Pregnancy Rate (%)	90	85	95	91
Maternal Wastage				
#Died (Pregnant)	0	0	0	4
#Non pregnant	2	3	1	2
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Corpora lutea	9	9	9	9
Implantations/Doe	8	9	9	9
Dead Fetuses/Doe	0	0	0	0
Live Fetuses/Doe	8	8	8	8
Resorptions/Doe	0	1	1	0

mean/litter

	5			
Total Resorbed Fetuses	5	17	15	17
Mean Fetal Weight (gm)	36	38.5	35	35.9
Postimplantation Loss(%)	3.6	10.0	8.8	4.6
Sex Ratio (% Male)	52.4	49.9	50.6	52.6

a = Data extracted from study report Table 5

None of the above parameters appeared to be affected by treatment.

2. Developmental Toxicity

Gross examination found one instance of each of the following abnormalities in the control group: unilateral microphthalmia, harelip and microglossia. Two kinked tails were observed in one litter from the mid-dose group.

Sectioning of the head found a single cleft palate and a hemorrhage of the vitreous humor, both in the control group. Visceral examination found four fetuses with enlarged auricles from one litter, three fetuses from one litter with ascites, and one fetus with an accessory left subclavian artery, all in the mid-dose group. "Left carotid artery arising from innominate" was found in 6 fetuses from 2 litters in the high dose group and a single fetus in the control group. Table 4 presents selected findings from the skeletal examination.

Table 4. Skeletal Examinations

<u>Observations</u>	<u>Control</u>	<u>Low Dose</u>	<u>Mid Dose</u>	<u>High Dose</u>
#pups(litters) examined	141(18)	129(17)	150(19)	167(20)
spine bent-bilateral	0	0	7(1)	0
unossified sternebrae	20(12)	27(9)	31(13)	38(15)
misaligned sternebrae	1(1)	0	0	0
extra ribs	33(14)	42(12)	36(13)	47(13)
#pups (litters) examined	64(18)	60(17)	69(19)	79(20)
unossified hyoid	1(1)	3(3)	2(2)	2(2)
accessary skull bone	3(3)	4(4)	7(5)	5(4)

None of the external, soft tissue or skeletal observations are considered to be treatment related.

D. Discussion/Conclusions

a. Maternal Toxicity: Pronounced maternal toxicity in the forms of clinical signs and decreased body weight gain were

observed at the high dose level. Maternal toxicity may have contributed to the 4 deaths at the high dose level.

b. Developmental Toxicity:

- i. Deaths/Resorptions: No effect under conditions of test.
- ii. Altered Growth: No effect on growth was observed at any dose level.
- iii. Developmental Anomalies: No developmental anomalies were observed which are considered to be treatment related.
- iv. Malformations: No effect on the induction of malformations under the conditions of the test.

D. Study Deficiencies: Food consumption was not measured.

E. Core Classification: Core minimum

Maternal NOEL = 0.7 mg/kg/day

Maternal LOEL = 1.8 mg/kg/day

Developmental toxicity NOEL = 1.8 mg/kg/day (HDT)

Endosulfan

OECD 6

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

00148c

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE:

SUBJECT: Teratogenicity of FMC 5462 in Rabbits. (Acc No. 246792, Reg. No. 279-2306, Caswell No. 420)

FROM: George Z. Ghali, Ph.D. *G. Ghali*
Review Section IV
Toxicology Branch, HED (TS-769)

TO: George La Rocca
Product Manager No. 15
Registration Division (TS-767-C)

THRU: Christine F. Chaisson, Section Head *C. F. Chaisson*
Review Section IV
Toxicology Branch, HED (TS-769)

Registrant: FMC Corporation
Agricultural Chemical Group
Philadelphia, Pa. 19103

Action Requested:

Review and evaluation of a rabbit teratology study on FMC 5462 (Endosulfan).

Conclusion and Recommendations:

1. The test chemical is not teratogenic under the experimental conditions.
2. Maternal toxicity was evident at 1.8 mg/kg/day (HTD) as manifested by the labored breathing and tonic convulsions of pregnant animals in this group. The NOEL for maternal toxicity is considered to be 0.7 mg/kg/day.
3. The study is adequate and conforms to standard testing procedures as outlined in the proposed Guideline (1978) except for the fact that no positive control group was included and food and water consumptions were not reported.
4. This study is classified as Core-minimum.

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Testing Laboratory:

Raltech Scientific Services, Madison, Wisconsin. Report No. A-79-370, dated July 27, 1981.

Test Animal:

Young adult New Zealand white rabbits, obtained from Hoppers Unlimited, Verona, Wisconsin and acclimated for 4 weeks.

Procedure:

pregnant rabbits were given the test chemical at the rate of 0.3, 0.7 or 1.8 mg/0.5 ml corn oil/kg maternal body weight/day by oral gavage on days 6-28 of gestation. The control group received corn oil only. Each treatment group consisted of 20 mated rabbits. When mortality was observed at the highest dose level, six more mated rabbits were added to this group. Maternal weights were recorded at day 0 (first day of treatment) and at 6-day intervals thereafter and at the time of sacrifice. Animals were observed twice daily throughout the test period for any toxic signs, abnormalities in activity and appearance, morbidity and mortality. On day 29 of gestation, the dams in each group were sacrificed by euthanatization with carbon dioxide, and the entire reproductive tract was removed including both ovaries. Ovaries were examined for abnormalities and the number of corpora lutea was recorded. Uterus was examined, weighed and fetuses were removed.

The number of live and dead fetuses, early and late resorptions, implantation sites were recorded.

All viable fetuses were sexed, measured, weighed, grossly examined, and examined for visceral abnormalities. Freehand sections were made of the heads of one half of the fetuses to permit gross examination of the eyes, palate, nasal septum and brain. All fetuses were prepared for skeletal examination and evaluated for bone alignment, degree of ossification, and abnormalities.

Results:

A. Maternal Observations:

Four animals in the 1.8 mg/kg group died on gestation days 7, 10, 21 and 29. The first three are thought to be due to improper oral gavage. The probable cause of death for the last one was not established, but gross and histopathological examinations revealed a pale appearance of the liver and kidneys in addition to vacuolization of the hepatocytes. Two animals in the control and one in the middle dose level showed nasal congestion. In the highest dose level, four animals showed a noisy and rapid breathing, hyperactivity and convulsions.

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There were no statistically significant difference in group mean body weights between the treated and control groups throughout the duration of the experiment.

Ascites was observed in approximately equal proportions in all groups.

At necropsy, the number of pregnant animals were 18, 17, 19, and 24 in the control, low, middle and high dose respectively. All pregnant females had viable litters, with the exception of the animal in the high dose level which died on test and had nine late resorptions.

There were no statistically significant differences in the mean number of corpora lutea, implantation efficiency, litter size, sex ratio, mean fetal length and weight, or in the number and percent of live and resorbed fetuses. Furthermore, there were no dead fetuses in the treatment groups. Three of the four animals which died on test had normally developing implants at necropsy.

3. Fetal Data:

No gross external abnormalities were observed in any of the fetuses in any treatment group except for two fetuses from one litter in the middle dose group had a kinked tail.

Two fetuses from separate litters in the control group had cleft palate and hemorrhagic vitreous humor.

One fetus from the control group and six fetuses from two litter in the high dose group had the left carotid artery arising from innominate.

Four fetuses from one litter in the middle dose group had enlarged auricles, another fetus with an accessory left subclavian artery.

The authors stated that common skeletal variation and minor skeletal anomalies were present in all groups and included: bent scapular spine, unossified, misaligned, and fused sternbrae, rudimentary ribs, extra ribs, fused ribs, interrupted ossification of a rib, 27 presacral vertebrae, fused caudal vertebrae, and unossified tail. Examination of the data indicated that these variations and other minor anomalies occurred throughout all groups in a non-treatment related pattern. Other than that, no major skeletal malformations were observed in any group.

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Reviewed by: Linda L. Taylor, Ph.D.
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel
Section II Head, Tox. Branch II (H7509C)

K. Clark Swentzel for
K. Clark Swentzel

JUN 23 1992

JUN 23 1992

DATA EVALUATION REPORT

STUDY TYPE: 2-generation reproduction - rat TOX. CHEM. NO.: 420

MRID/TRID NO.: 148264/460002-031; Accession # 256127

TEST MATERIAL: Endosulfan technical

SYNONYMS: 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9,-
methano-2,4,3-benzodioxathi epin-3-oxide; HOE 02671 0 I
AT209

STUDY NUMBER: HST 204/83768

SPONSOR: Hoechst Celanese Corporation

TESTING FACILITY: Huntingdon Research Centre

TITLE OF REPORT: Effect of Endosulfan-Technical (Code 02671 0 I
AT209) on Reproductive Function in the Rat

AUTHORS: JA Edwards. YJ Reid, JM Offer, RH Almond, and WA Gibson

REPORT ISSUED: July 19, 1984

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, exposure to Endosulfan via the diet during pre-mating and through gestation and lactation, at dose levels of 0, 3, 15, and 75 ppm, produced minimal maternal toxicity at the high-dose level. Mortality, food/water consumption, and body weight were not affected in either generation, but there was a decrease in body-weight gain in the F0 females following the start of dosing. Pregnancy rate, gestation times, the ability to rear young to weaning, and pre-coital time were comparable among the groups at both matings in both generations. F0 males displayed increased heart weight at the mid- and high-dose levels (dose-related) and increased liver and kidney weights at the high-dose level. F0 females displayed increased brain and liver weights at the high-dose level. In the F1b adults, the high-dose males displayed increased kidney weights compared to the controls and females displayed increased liver weights at the mid- and high-dose levels. There was no effect of treatment on litter size throughout both matings of both generations. In the first mating of the F0 generation, there was an increase in the cumulative litter loss (%) at the high-dose level. Litter and pup weights were comparable at birth among the groups in both generations, but there was a decrease in litter weight observed during the lactation to weaning period in both matings in the F0

generation, which was significant at the high-dose in the first mating and at the mid- and high-dose levels in the second mating (dose-related). Because there was no corroborative finding of a decrease in the number of pups per litter or in pup weight, this decrease in litter weight is not considered to be treatment-related. Increased pituitary weights (high-dose ♀♀ pups of 1st mate in F0 generation) and increased uterine weights (high-dose ♀♀ pups of 1st mate of F1b generation) were observed in the offspring. There were no histopathological findings observed in either the F1b adults or the selected pups from the second mate of the F1b generation that could be attributed to treatment.

The NOEL for maternal toxicity can be set at 3 ppm (≈ 0.2 mg/kg/day), the LEL at 15 ppm (≈ 1.0 mg/kg/day), based on increased liver weight. A NOEL for reproductive effects can be set at 75 ppm (≈ 5 mg/kg/day), the highest dose tested. Although there were no significant effects noted on the dams, the dose levels are considered adequate, based on the results of the range-finding study in which there was an increase in cumulative pup loss and a reduction in litter size at the 100 ppm dose level at days 24 and 28 post weaning. The NOEL for effects on the offspring can be set at 15 ppm, the LEL at 75 ppm, based on increased pituitary and uterine weights.

Classification: Core: Minimum. This study satisfies the guideline requirements (83-4) for a 2-generation reproduction study.

A. MATERIALS

1. Test Compound: Endosulfan technical; Description: not provided; Batch #: HOE 02671 O I AT209; Purity: 97%.
2. Test Animals: Species: rat; Strain: Cr1: COBS CD⁰(SD) BR; Age: 6 weeks old; Weight: ≈31 grams on arrival; Source: Charles River, UK Ltd., Margate, Kent.
3. Statistics: Weekly adult body weights - analysis of variance; Litter data - non-parametric tests (Jonckheere and Kruskal-Wallis); Organ weight - analysis of variance or covariance (final body weight as covariant when the within-group relationship between organ weight and body weight was significant at the 10% level. A log (x+1) transformation of organ weight (or of both body weight and organ weight) was used if significant (1% level) heterogeneity of variance was revealed in the organ weight (body weight) data by Bartlett's test, and if the transformed data showed less heterogeneity of variance than the untransformed data. Intergroup comparisons were carried out using Williams' test.

B. STUDY DESIGN

1. Methodology: On arrival, an unspecified number of male and female rats were weighed and examined for abnormalities/signs of ill health and, following a 7-day quarantine period, were weighed again and assigned to four groups by computerized stratified randomization to give approximately equal initial mean body weights. Treatment began following a second 7-day acclimation period. There were 32 rats/sex/group in the F0 generation and 28 rats/sex/group in the F1 generation. The test material was incorporated into the diet at fixed concentrations of 0, 3, 15, and 75 ppm and fed to both sexes throughout two matings/generation for two consecutive generations. During the pre-mating periods, the animals were housed 4/cage (sexes separately), with male cages interspersed among the female cages to promote development of regular estrous cycles. During the mating period (20 days), one male and one female were housed together in plastic breeding cages. At the end of the mating period, the males were returned to their original cages, and the females were housed in individual breeding cages for the birth and rearing of their young. Suitable nesting material was provided. Feed (Spratt's Laboratory Diet No. 2; powdered diet) and tap water were available ad libitum.

F0 Generation: Exposure of the F0 animals (32 rats/sex/group) to the test material via the diet (0, 3, 15, 75 ppm Endosulfan technical) began when they were 6 weeks of age and continued for 84 days, at which time (≈18 weeks of age) they were mated [one ♂ with one ♀ for 20 days]. Exposure continued until all litters were weaned. The F0 dams were allowed to rear their young to Day 21 post partum, at which time the F1a young were

sacrificed [for 1 pup/sex/litter, specified organs were weighed and tissues preserved for possible future examination; see under 3(e), below]. Approximately 10 days after weaning the F1a pups, F0 males and females were re-mated (different mates; those not pregnant previously and males failing to induce pregnancy were mated to those that were successful at the first mating). As before, the animals were mated for a 20-day period, and the dams were allowed to rear their young to day 21 post partum. At day 21 post partum, 28 pups/sex/group were selected to form the basis of the F1b generation. One pup/sex was chosen (close to median weaning weight/sex) from each of 28 litters, where possible [if < 28 litters available, a second pup from a litter was chosen; where > 28 litters available, those (litters) deviating least from the median weaning weight were used]. Excess pups were sacrificed, and one pup/sex/litter had specified organs weighed and tissues preserved [see 3(e), below]. Shortly after the F1b pups were weaned, the F0 parents were sacrificed and specified organs were weighed and tissues were preserved for possible histopathological examination [see 3(e), below].

F1b Generation: The selected F1b pups were reared on their respective diets for at least 98 days prior to mating (\approx 18 weeks old), and they were mated as described above. Sibling pairings were avoided. Day 21 post partum, or soon after, the F2a pups were sacrificed and processed as were F1a pups. After \approx 10 days, the F1b animals were re-mated, as described for F0 animals above. The females were allowed to rear their young to day 21 post partum, at which time all F2b pups and F1b adults were sacrificed. Specified organs were weighed and a full range of tissues were preserved for one pup/sex/litter and all F1b adults. Tissues of the adults and the selected pups from the control and high-dose groups were subjected to histopathological examination [see 3(e), below]. Additionally, testes and accessory organs of all males failing to induce pregnancy at the second mate and ovaries of females without young at the second mate were examined histologically.

- 2. Dose preparation:** The test material was weighed out and ground, dissolved in a small volume of acetone, and corn oil was added. This mixture was added to a quantity of sieved diet and stirred thoroughly. After evaporating off the acetone, additional diet was added to give a pre-mix of suitable strength. The dietary concentrations required were obtained from this pre-mix by direct dilution with additional diet and further mixing. The diets were prepared freshly every two weeks and stored at \approx 4°C. The homogeneity and stability of Endosulfan in the diet were established in a preliminary study (HST 203/82253; DER dated 10/31/85); the concentrations achieved in this study were measured during each generation at the start of the pre-mating treatment period, at the start of each mating period, and shortly after each mating period.

RESULTS

The mean concentrations of Endosulfan in the dose formulations analyzed during the study were all within 10% of the nominal concentrations.

Because the dietary levels of Endosulfan were maintained at constant levels throughout the study, the achieved intake of the test material (mg/kg/day) decreased in the parental animals (both generations) as the animals grew. The table below lists the achieved intakes for the F0 and F1b generations.

Week Group*	MALES			FEMALES		
	3	15	75	3	15	75
F0 generation						
1	0.32	1.61	7.96	0.34	1.65	7.55
2	0.28	1.39	6.93	0.31	1.66	8.00
3	0.24	1.21	6.00	0.29	1.46	7.46
4	0.22	1.13	5.61	0.28	1.43	7.02
5	0.20	0.99	4.99	0.24	1.21	6.32
6	0.18	0.94	4.69	0.23	1.25	6.62
7	0.18	0.87	4.43	0.22	1.12	5.75
8	0.16	0.82	4.12	0.21	1.07	5.46
9	0.16	0.78	3.97	0.20	1.01	5.35
10	0.15	0.75	3.84	0.19	1.04	5.03
11	0.15	0.76	3.80	0.18	0.93	4.87
12	0.14	0.71	3.59	0.18	0.92	4.71
Overall	0.20	1.00	4.99	0.24	1.23	6.18
F1b generation						
5	0.45	2.38	11.01	0.47	2.27	11.54
6	0.37	1.92	9.49	0.37	1.81	9.83
7	0.31	1.60	7.70	0.32	1.69	8.70
8	0.27	1.38	6.70	0.29	1.49	7.48
9	0.24	1.20	5.95	0.27	1.33	7.59
10	0.22	1.11	5.39	0.25	1.28	6.56
11	0.20	1.01	5.08	0.25	1.27	6.61
12	0.19	0.95	4.63	0.23	1.13	6.01
13	0.18	0.92	4.45	0.22	1.13	6.27
14	0.17	0.89	4.27	0.21	1.09	5.71
15	0.16	0.86	4.01	0.21	1.08	5.48
16	0.16	0.82	3.95	0.19	1.01	5.22
17	0.15	0.77	3.75	0.18	0.96	5.01
18	0.14	0.76	3.66	0.18	0.95	4.85
Overall	0.23	1.18	5.72	0.26	1.32	6.92

* ppm

3. Parental Investigations

- (a) Clinical Observations: All animals were handled regularly and examined for obvious changes or signs of reaction to treatment. Animals showing marked signs of ill health were sacrificed to prevent cannibalism or autolytic degeneration. All animals dying on test or sacrificed were weighed and subjected to post mortem examination to establish cause of death.

RESULTS

Survival and Clinical Observations: There were 4 deaths during the study: F0 Generation - 1 control, 1 low-, and 1 mid-dose female died. F1b Generation - 1 control female died. The deaths were not considered to be related to treatment. Clinical signs were comparable among the groups in both generations.

- (b) Food and Water Consumption: Food intake was recorded weekly throughout the pre-mating phases, and food conversion ratios/mg/kg/day intake of test material were calculated. Water consumption was measured on a daily basis during the initial two and final two weeks of the pre-mating treatment periods for each generation.

RESULTS

Pre-mating period: F0 Generation - Food consumption was comparable among the groups during both pre-mating periods. Water consumption varied among the groups, but there was no apparent relationship to treatment. F1b Generation - Males at the 75 ppm dose level displayed slightly lower (91-95% of control value) food consumption values compared to the controls throughout the pre-mating period. Water intake was comparable among the groups.

- (c) Body Weight: All animals were weighed, initially at 5 weeks of age for F0 generation and at selection for F1b generation, at weekly intervals. During the mating period, all females were weighed on alternate days throughout. Weights are reported for Days 0 (day of occurrence of a positive indication of mating; i.e., sperm or plug), 7, 14, 17, and 20 of gestation. Weights of pregnant animals without a positive indication of mating are reported for appropriate days taken retrospectively from birth. Dams that littered were weighed on Days 0, 7, 14, and 21 post partum.

RESULTS

F0 Generation: The only effect observed was a slight decrease in body weight at the highest dose level in females compared to the control values. During the first week of treatment, the body-weight gain for the high-dose females was 67% of the control value; the body-weight gain during the first 4 weeks was 91% of control value. The overall body-weight gain during the pre-mating period was 96% of the control value in the high-dose females.

F0 Generation Group Mean Body Weight

Week	Males (% of Control)			Females (% of Control)		
	Low	Mid	High	Low	Mid	High
0	101	101	101	100	100	100
1*	103	101	101	100	99	96
2	103	102	102	99	98	96
3	104	102	102	100	99	96
6	105	103	103	100	100	98
12*	104	101	102	100	99	98
15	103	103	101	98	96	96
16	104	101	102	98	96	97
17	105	101	103	99	97	98
21*	105	101	103	100	100	97
23	105	100	102	99	98	97
24	105	99	102	98	96	96
29	105	100	103	102	101	99
BWG 0-1*	107*	104	102	100	96	67***
BWG 0-4*	108**	105	102	100	96	91*
BWG 0-12*	106	102	103	100	99	96
BWG 12-15	-	-	-	91	86	87
BWG 19-20	-	-	-	114	93	71
BWG 20-21	-	-	-	100	100	83
BWG 21-24	-	-	-	92	84	93

* p<0.05; ** p<0.01; *** p<0.001; * start of pre-mating period; * start of mating period; BWG - body-weight gain; BWG* first pre-mating period; (-) not calculated for males; NOTE: statistics not performed on BWG data for 12-15, 19-20, 20-21, 21-24.

F1b Generation: The body weights of all treatment groups (both sexes) were lower than their respective control values at week 4 (dose-related in males); this relationship to control remained throughout the remainder of the study, with the females displaying a dose response by week 5. After week 4, all treated groups displayed values $\geq 90\%$ of the control value. There were no differences noted in body-weight gain in either sex.

F1b Generation Group Mean Body Weight (% of Control)

Week	Males (% of Control)			Females (% of Control)		
	Low	Mid	High	Low	Mid	High
4*	94	91	89	88	95	95
5	98	96	92	96	96	94
17	101	97	96	103	100	98
18*	101	97	95	103	99	97
21	101	97	95	101	101	97
27*	103	97	96	102	100	98
31	102	98	96	103	101	100
38	102	96	95	101	100	98
BWG 4-18	102	98	97	108	101	99
BWG 18-27	113	100	99	93	104	100

* p<0.05; ** p<0.01; † start of pre-mating period; ‡ start of mating period; † gestation; ‡ lactation
 BWG - body-weight gain: BWG

Gestation - Body weight was comparable among the groups throughout gestation in both generations (both matings). Body-weight gain was decreased at the low- and high-dose levels in the F0 dams during gestation following the first mating at various intervals, but was comparable among the groups after the second mating. In the F1b generation, body-weight gain was comparable among the groups after the first mating and greater in the treated dams compared to the controls after the second mating.

Body-Weight Change During Gestation

Interval (days)	F0 Body Weight Change (% of Control)			F1b Body Weight Change (% of Control)		
	Low	Mid	High	Low	Mid	High
FIRST MATE 0-7	85	92	77	110	114	110
7-14	91	94	88	100	100	92
0-14	88	93	83	104	106	96
0-20	100	102	95	102	107	95
SECOND MATE 0-7	92	75	96	112	129	106
7-14	100	86	100	112	108	112
0-14	96	81	98	112	117	110
0-20	103	95	98	110	116	107

Lactation - Body weight and body-weight change were comparable among the groups (both matings/both generations), displaying a similar relationship as was found during gestation.

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(d) Pregnancy Rate, Mating Performance, and Gestational Period

The pregnancy rate was determined as the percent of surviving paired females that became pregnant. With regard to mating performance, vaginal smears were taken daily during the 20-day mating period to enable the number of animals that mated on a specific day to be determined in order to (1) detect whether pregnancy was interrupted after mating; (2) detect marked anomalies of the estrus cycle; and (3) determine the median pre-coital time for the group. The gestation period for females that littered was taken as the time between the day of successful mating and parturition.

RESULTS

F0 Generation: The median pre-coital times (both matings) were comparable among the groups. Pregnancy rates were comparable among the groups after both matings, although the low- and mid-dose groups displayed the lowest rates each time. Gestation times and the number rearing their young to weaning were comparable among the groups.

F1b Generation: No differences were observed in the median pre-coital times, pregnancy rates, gestation times, and the number rearing their young to weaning among the groups (both matings) that could be attributed to treatment.

Median Pre-coital Time (days)*

Generation/Group	Control	Low	Mid	High
F0				
1st mating	3.0	3.0	3.0	3.0
2nd mating	2.5	3.0	2.0	3.0
F1b				
1st mating	3.0	3.0	3.0	3.0
2nd mating	3.0	3.0	3.0	3.0

*day by which half of the females successfully paired had conceived

Pregnancy Rate (%)

Generation/Group	Control	Low	Mid	High
F0				
1st mating	97	91	84	97
2nd mating	94	88	84	94
F1b				
1st mating	96	93	89	93
2nd mating	96	96	86	93

(e) Sacrifice and Pathology

Parent Animals: Shortly after the pups from the second mate litters were weaned, the parental animals were sacrificed and examined macroscopically. The following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, testes with

epididymides, and pituitary [for males failing to induce pregnancy at the second mate, the prostate gland and seminal vesicles were weighed also; the uteri of apparently non-pregnant females were examined by the Salewski technique]. Additionally, a full range of tissues [CHECKED (X)] was preserved from all animals; histopathological examination was limited to F1b adults from the control and high-dose groups.

Digestive system		Cardiovasc./Hemat.	Neurologic		
X	Tongue	X	Aorta	X	Brain
X	Salivary glands	X	Heart	X	Periph. nerve (sciatic)
X	Esophagus	X	Bone marrow	X	Spinal column
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes
X	Jejunum	X	Thymus		Glandular
X	Ileum		Urogenital	X	Adrenal gland
X	Cecum	X	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
	Rectum	X	Testes		Parathyroids
X	Liver	X	Epididymides	X	Thyroids
	Gall bladder	X	Prostate		Other
X	Pancreas	X	Seminal vesicle	X	Bone
	Respiratory	X	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung		Cervix	X	All gross lesions and masses
	Nasal turbinates		Vagina		
	Pharynx				
	Larynx				

Additionally, the testes, prostate, and seminal vesicles of all males failing to induce pregnancy at the second mate and ovaries of females without young at the second mate were examined histologically for both generations.

RESULTS

Gross Pathology: F0 Adults - Enlarged livers and kidneys were observed in greater numbers in the high-dose F0 males compared to the control groups. There were no apparent differences in the incidences of macroscopic changes in the low- and mid-dose males or in any of the female groups compared to their respective controls.

F1b Adults - There were no clear treatment-related changes in the incidences of macroscopic lesions among any of the groups in either sex.

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Macroscopic Findings in Adult Males at Necropsy

Macroscopic Finding/ Generation/Dose group*	F0 Generation				F1b Generation			
	0	3	15	75	0	3	15	75
<u>kidneys</u> - males N= enlarged	32 3	32 3	32 3	32 7	28 0	28 0	28 0	28 0
<u>liver</u> - males N= enlarged	32 4	32 5	32 5	32 10	28 0	28 1	28 0	28 0
median cleft accessory lobe	nr	nr	nr	nr	5	4	6	8
median cleft accessory lobe w/ pale subcapsular area	nr	nr	nr	nr	2	3	0	1
median cleft w/ pale subcap. area	nr	nr	nr	nr	3	0	1	4
minimally swollen	nr	nr	nr	nr	2	1	0	2

* ppm; nr not reported

Macroscopic Findings in Adult Females at Necropsy

Macroscopic Finding/ Generation/Dose group*	F0 Generation				F1b Generation			
	0	3	15	75	0	3	15	75
<u>kidneys</u> - females N= enlarged	32 0	32 0	32 0	32 0	28 0	28 0	28 0	28 0
<u>liver</u> - females N= enlarged	32 0	32 0	32 0	32 2	28 0	28 0	28 0	28 0
median cleft accessory lobe	nr	nr	nr	nr	0	0	0	0
median cleft accessory lobe w/ pale subcapsular area	nr	nr	nr	nr	0	0	0	0
median cleft w/ pale subcap. area	nr	nr	nr	nr	0	0	2	1
minimally swollen	nr	nr	nr	nr	0	0	0	0

* ppm; nr not reported

Organ Weights: Adults: Liver weight was increased at the 75 ppm dose level in F0 generation males and females and at the 15 and 75 ppm dose levels in F1b generation females. Kidney weight was increased at the high-dose level in males in both generations. Heart weights were increased in the mid- and high-dose males (dose-related) in the F0 generation only, and the brain weight in the high-dose females in the F0 generation was slightly increased.

MALES (Adults)

Organ Weight Data (% Control)

Dose (PPM)	F0 Generation			F1b Generation		
	3 ppm	15 ppm	75 ppm	3 ppm	15 ppm	75 ppm
Organ Weights						
BRAIN	101 (101)	101 (101)	101 (101)	100 (100)	98 (98)	99 (99)
KIDNEY	105 (102)	103 (103)	113 (111)**	103 (100)	101 (102)	110 (112)**
LIVER	104 (99)	103 (103)	110 (107)*	105 (101)	96 (98)	101 (104)
HEART	107 (104)	104 (105)*	109 (107)**	99 (97)	100 (101)	97 (98)
final body weight	105	100	103	105	98	97

* () value adjusted for body weight; relates to organs where the within-group relationship to body weight was significant at $p < 0.01$; analysis of covariance using final body weight as covariate, followed by Williams' test

FEMALES (Adults)

Organ Weight Data (% Control)

Dose (PPM)	F0 Generation			F1b Generation		
	3 ppm	15 ppm	75 ppm	3 ppm	15 ppm	75 ppm
Organ Weights*						
BRAIN	100 (100)	100 (101)	102 (102)*	98 (98)	99 (99)	101 (101)
KIDNEY	98 (99)	97 (98)	100 (103)	102 (101)	96 (96)	106 (105)
LIVER	100 (101)	98 (100)	107 (110)**	104 (102)	107 (108)**	113 (112)***
HEART	99 (99)	97 (99)	99 (101)	109 (107)	101 (101)	102 (101)
final body weight	99	97	96	102	99	101

* () value adjusted for body weight; relates to organs where the within-group relationship to body weight was significant at $p < 0.01$; analysis of covariance using final body weight as covariate, followed by Williams' test

Histopathology: Adults - No significant changes were observed in either sex in either generation. The discoloration observed in the kidneys is attributed to the presence of the test material (see RfD memo dated 5/15/92).

4. Offspring Investigations

- (a) All litters: As soon after parturition as possible, the pups were counted, individually identified within the litter by toe amputation, sexed, weighed, and examined for external abnormalities. With minimal nest disturbance, all litters were examined daily for dead and/or abnormal pups, and pups were weighed on days 4, 8, 12, and 21 post partum. **F0 Generation** - The total number of offspring was determined at birth, and the number of live pups/litter was determined at birth and at day 4, 8, 12, and 21 post partum. Litter and mean pup weights were calculated from the individual pup weights. Pup mortality was determined at birth and cumulatively on days 4, 8, 12, and 21 post partum ($\frac{\# \text{ of pups born} - \# \text{ of pups surviving}}{\# \text{ of pups born}} \times 100$)

Where possible, dead pups were necropsied. On or shortly after Day 21, excess pups were sacrificed and examined macroscopically, with any abnormal tissue being preserved. Sex of each pup was confirmed by gonadal inspection. For one pup/sex/litter (selected on the basis of median body weight), specified organs were weighed and tissues preserved for possible histopathological examination [see above under 3(e)]. Surviving pups were weighed on Days 4, 8, 12, and 21 post partum. At Day 21 post partum, 28 pups/sex/group were selected to form the basis of the F1b generation. **F1b Generation** - Same as above, except no pups were selected to form another generation.

NOTE: The assessment of litter parameters was calculated two ways by the authors. **Mean A** - includes all valid data from surviving animals that provide evidence of pregnancy, including those subsequently losing the entire litter (failing to wean young). **Mean B** - includes all valid data from any animal with young surviving to weaning. Additionally, animals showing direct evidence of pregnancy only by the presence of implantation sites as revealed by the Salewski technique, were not included in the calculation of mean values. Mean A values are affected by the loss of whole litters, which is often due to maternal

neglect or effects upon the parent animal; Mean B values exclude such losses and give an indication of any general effect on all young. For litter and mean pup weights and abnormality values, only Mean B values were calculated by the authors.

RESULTS

F0 Generation - There were no apparent adverse effects of treatment on litter size or litter/pup weight at birth at either mating. Decreased litter weight was displayed at the high-dose level at day 8 through 21 in both matings, and the mid-dose level also displayed a decrease in the second mating from day 4 on, although statistical significance was not attained at either dose level at day 21 in the second mating. There was a slight increase in cumulative pup loss at the mid- and high-dose levels at the first mating only. At weaning, litter size was comparable among the groups at both matings. Additionally, there was no effect on the sex ratio.

F1b Generation - No adverse effects were observed on any of the parameters measured at either mating.

FO LITTER DATA (1st mating) †

PARAMETER	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
# implants	ND	ND	ND	ND
Pre-birth loss (%)	ND	ND	ND	ND
Litter size (live) †	11.6	12.5	12.7	11.8
% pup loss	0.7	0.5	1.0	1.7
Litter weight †	67.4	72.3	72.6	68.4
pup weight †	6.0	5.9	5.7	5.9
sex ratio ♂/♀ birth	6.1/5.6	6.3/6.3	6.5/6.3	6.0/6.0
sex ratio ♂/♀ day 21	5.9/5.5	6.1/6.0	6.0/6.0	5.5/5.5
LITTER SIZE				
Day 4	11.5	12.3	12.6	11.5
Day 8	11.4	12.3	12.4	11.4
Day 12	11.3	12.2	12.3	11.1
Day 21	11.2	12.1	12.0	11.1
LITTER WEIGHT				
Day 4	104.6	107.5	106.2	100.3
Day 8	170.3	174.7	174.5	157.6*
Day 12	235.8	245.9	243.3	218.4*
Day 21	434.4	449.4	436.5	392.6*
MEAN PUP WEIGHT				
Day 4	9.5	8.9	8.5	9.2
Day 8	15.6	14.6	14.1	14.6
Day 12	21.7	20.7	19.8	20.9
Day 21	40.2	38.0	36.6	38.3
CUMULATIVE LOSS (%)				
Day 4	2.4	1.9	2.1	4.4
Day 8	3.1	2.2	3.6	5.0
Day 12	4.1	2.7	3.9	7.1
Day 21	4.4	3.3	6.7	7.8

† data presented as Mean B values or as Mean A/Mean B values; † at birth; ND not determined

FO LITTER DATA (2nd mating) ♦

PARAMETER	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
# implants	ND	ND	ND	ND
Pre-birth loss (%)	ND	ND	ND	ND
litter size (live)♦	12.4/12.8	12.6/13.0	12.3/12.6	12.0
% pup loss♦	0.8/0.9	3.5/1.1	1.8/0.9	1.6
litter weight♦	76.1	74.7	73.4	73.4
pup weight♦	6.0	5.8	5.9	6.2
sex ratio ♂/♀ birth	6.7/6.2	6.4/6.8	6.2/6.6	6.4/5.8
sex ratio ♂/♀ day 21	6.5/6.0	6.0/6.3	5.9/6.4	5.9/5.6
<u>LITTER SIZE</u>				
Day 4	12.3/12.7	12.0/12.5	12.0/12.5	11.9
Day 8	12.1/12.5	12.0/12.4	11.8/12.3	11.8
Day 12	12.1/12.5	11.9/12.3	11.8/12.3	11.7
Day 21	12.1/12.5	11.9/12.3	11.8/12.3	11.5
<u>LITTER WEIGHT</u>				
Day 4	121.0	115.8	113.0*	112.1
Day 8	191.2	185.6	177.6*	172.5*
Day 12	272.7	264.8	252.8*	240.8**
Day 21	491.3	483.5	468.6	443.8
<u>MEAN PUP WEIGHT</u>				
Day 4	9.8	9.4	9.2	9.7
Day 8	15.7	15.3	14.7	15.0
Day 12	22.4	22.1	20.8	21.2
Day 21	40.3	40.5	38.6	39.4
<u>CUMULATIVE LOSS (%)</u>				
Day 4	1.8/1.8	8.3/4.9	5.2/1.4	2.8
Day 8	3.1/3.2	8.6/5.2	6.5/2.8	3.3
Day 12	6.5/3.2	9.3/5.9	6.5/2.8	4.1
Day 21	6.5/3.2	9.6/6.3	6.5/2.8	5.8

♦ data presented as Mean B values or as Mean A/Mean B values; ♦ at birth; ND not determined

F1b LITTER DATA (1st mating) ♦

PARAMETER	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
# implants	ND	ND	ND	ND
Pre-birth loss (%)	ND	ND	ND	ND
litter size♦	12.1	11.8/11.8	13.1/13.0	11.4/11.6
% pup loss♦	0.9	0.9/1.0	0.9/0.9	0.7/0.3
litter weight♦	65.3	64.8	71.0	63.1
pup weight♦	5.5	5.6	5.6	5.5
sex ratio ♂/♀ birth	5.6/6.6	5.7/6.2	6.8/6.3	5.3/6.3
sex ratio ♂/♀ day 21	5.3/5.9	5.4/5.4	5.8/5.5	4.8/5.4
<u>LITTER SIZE</u>				
Day 4	11.6	11.6/11.6	12.8/12.7	11.0/11.2
Day 8	11.4	11.1/11.1	12.4/12.3	10.8/10.9
Day 12	11.3	10.7/10.8	11.8/11.7	10.5/10.6
Day 21	11.2	10.4/10.8	10.8/11.3	9.9/10.3
<u>LITTER WEIGHT</u>				
Day 4	82.8	89.6	90.5*	82.0
Day 8	125.2	141.3*	134.8	124.0
Day 12	187.5	205.4	200.1	177.7
Day 21	355.0	405.6	376.9	339.9
<u>MEAN PUP WEIGHT</u>				
Day 4	7.3	8.0*	7.4	7.4
Day 8	11.3	13.3*	11.7	11.4
Day 12	17.1	19.8*	17.8	16.8
Day 21	32.8	39.1*	35.0	33.2

PARAMETER	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
CUMULATIVE LOSS (%)				
Day 4	6.0	2.3/2.1	3.0/3.1	3.7/3.4
Day 8	7.1	6.2/5.8	5.7/5.9	5.4/5.2
Day 12	8.1	8.8/8.0	9.0/9.4	7.9/7.7
Day 21	8.6	11.5/8.0	15.7/12.2	13.8/10.4

F1b LITTER DATA (2nd mating) *

PARAMETER	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
# implants	ND	ND	ND	ND
Pre-birth loss (%)	ND	ND	ND	ND
litter size*	11.9/12.3	11.7/11.7	13.5	12.5
% pup losses	1.7/1.7	1.5/1.2	0	1.1
litter weights	67.6	64.2	73.5	68.3
pup weights	5.5	5.6	5.5	5.5
sex ratio ♂/♀ birth	6.7/5.9	5.8/6.1	7.2/6.3	6.1/6.6
sex ratio ♂/♀ day 21	6.2/5.4	5.4/5.5	6.6/5.8	5.6/5.8
LITTER SIZE				
Day 4	11.3/11.8	11.1/11.5	13.1	12.1
Day 8	11.2/11.7	10.7/11.1	12.5	11.9
Day 12	11.2/11.6	10.6/11.0	12.4	11.5
Day 21	11.2/11.6	10.5/10.9	12.4	11.4
LITTER WEIGHT				
Day 4	93.1	91.0	100.9	91.2
Day 8	149.0	147.9	159.3	143.1
Day 12	232.8	229.7	248.1	222.3
Day 21	452.3	447.1	470.7	425.5
MEAN PUP WEIGHT				
Day 4	8.0	8.2	7.8	7.8
Day 8	13.0	14.1	12.8	12.6
Day 12	20.4	21.7	20.0	20.1
Day 21	39.6	42.4	38.5	38.9
CUMULATIVE LOSS (%)				
Day 4	9.7/6.1	6.2/2.6	2.7	4.5
Day 8	10.7/7.2	9.0/5.5	6.7	5.7
Day 12	11.0/7.4	9.7/6.3	7.6	8.3
Day 21	11.0/7.4	10.2/6.8	7.9	9.5

* data presented as Mean B values or Mean A/Mean B Values; * at birth; ND not determined; * p<0.05; ** p<0.01

- (b) **Terminal studies: Offspring:** The selected offspring were subjected to necropsy, the same organs listed above for the parents (plus the thymus) were weighed, and the same organs were preserved as were for the parent animals. The histological examination was limited to the control and high-dose pups selected from the second mate of the F1b generation.

RESULTS

Pituitary weights were increased in females from the first mating of the F0 generation at the 75 ppm dose level, and females from the first mating of the F1b generation displayed increased uterine weights at this same dose level. No other differences were reported, although the pituitary weight of the mid- and high-dose level pups from the second mating of the F1b generation were lower than the control values. Microscopic examination did not reveal any differences among the groups.

MALES (Offspring at Weaning)

Organ Weight Data (% Control)

Dose (PPM)	F0 Generation (1st mating)			F0 Generation (2nd mating)		
	3 ppm	15 ppm	75 ppm	3 ppm	15 ppm	75 ppm
Organ Weight*						
BRAIN	101 (102)	98 (100)	99 (101)	99 (99)	100 (101)	100 (101)
KIDNEY	98 (102)	95 (103)	96 (101)	97 (99)	90 (94)	96 (101)
LIVER	94 (99)	94 (103)	97 (103)	94 (95)	91 (96)	97 (103)
HEART	97 (101)	94 (102)	96 (101)	98 (99)	95 (101)	97 (102)
PITUITARY	97 (100)	106 (109)	91 (94)	103 (100)	123 (126)	109 (113)
final body weight	96	91	95	99	94	94

* () value adjusted for body weight; relates to organs where the within-group relationship to body weight was significant at $p < 0.01$;

MALES (Offspring at Weaning)

Organ Weight Data (% Control)

Dose (PPM)	F1b Generation (1st mating)			F1b Generation (2nd mating)		
	3 ppm	15 ppm	75 ppm	3 ppm	15 ppm	75 ppm
Organ Weight*						
BRAIN	102 (97)	101 (100)	98 (98)	100 (99)	100 (100)	99 (99)
KIDNEY	116 (97)	101 (96)	99 (99)	105 (98)	94 (95)	101 (102)
LIVER	120 (97)	97 (91)	97 (97)	108 (100)	98 (99)	103 (105)
final body weight	120	105	100	107	99	98

* () value adjusted for body weight; relates to organs where the within-group relationship to body weight was significant at $p < 0.01$;

FEMALES (Offspring at Weaning)

Organ Weight Data (% Control)

Dose (PPM)	F0 Generation (1st mating)			F0 Generation (2nd mating)		
	3 ppm	15 ppm	75 ppm	3 ppm	15 ppm	75 ppm
Organ Weight*						
BRAIN	96 (98)	99 (101)	97 (99)	99 (99)	96 (97)	98 (99)
KIDNEY	94 (102)	93 (100)	98 (103)	99 (100)	95 (100)	96 (102)
LIVER	90 (98)	95 (103)	98 (104)	96 (98)	94 (100)	95 (102)
PITUITARY	97 (100)	76 (78)	124 (128)*	109	94	97
UTERUS	109 (116)	107 (114)	100 (105)	104 (104)	106 (111)	86 (92)
final body weight	92	92	94	99	95	94

* () value adjusted for body weight; relates to organs where the within-group relationship to body weight was significant at $p < 0.01$;

FEMALES (Offspring at Weaning)

Organ Weight Data (% Control)

Dose (PPM)	F1b Generation (1st mating)			F1b Generation (2nd mating)		
	3 ppm	15 ppm	75 ppm	3 ppm	15 ppm	75 ppm
Organ Weight*						
BRAIN	102 (97)	101 (99)	100 (100)	101 (99)	99 (100)	98 (98)
KIDNEY	121 (103)	104 (98)	102 (101)	111 (101)	95 (99)	101 (103)
LIVER	125 (102)	105 (98)	105 (104)	113 (101)	96 (100)	100 (102)
PITUITARY	124 (106)	117 (110)	110 (106)	93	79	77
UTERUS	128 (111)	111 (106)	122 (121)**	107 (100)	100 (104)	93 (94)

* () value adjusted for body weight; relates to organs where the within-group relationship to body weight was significant at $p < 0.01$;

C. DISCUSSION

There was no evidence of maternal toxicity at any dose level; only a slight decrease in body-weight change was observed in the F0 females at the high dose during the first few weeks of treatment and during the first mating in the low- and high-dose F0 females. Grossly, enlarged livers and kidneys were observed in greater numbers in the high-dose F0 males than in control males, which correlate with the increases noted in the weights of these organs. There were several differences noted in organ weights, including (1) a dose-related increase in heart weight in the mid- and high-dose F0 males, (2) an increase in kidney and liver weights in the high-dose F0 males, (3) an increase in kidney weights in the high-dose F1b males, (4) an increase in liver weights in the high-dose F0 females and in the mid- and high-dose F1b females (dose-related), and (5) an increase in brain weight in the high-dose F0 females. Cumulative litter loss (%) was increased at the high-dose level in the first mating of the F0 generation. Decreased litter weight (dose-related) was observed at the mid- [2nd mating] and high- [1st and 2nd matings] dose levels in litters of the F0 generation, but with no corroborative decreases in pup weight and/or litter size, the finding is considered an artifact. In the offspring, increased pituitary weights were observed at the high-dose level in female pups from the first mating of the F0 generation, and increased uterine weights were displayed in female pups of the first mating of the F1b generation. There were no differences noted between the control and high-dose F1b adults and F2b weanlings with regard to the microscopic examination of the selected tissues.

D. CONCLUSION

Under the conditions of the study, exposure to Endosulfan technical via the diet during pre-mating (F0 generation - 84 days; F1b generation - 98 days) and through gestation and lactation, at dose levels of 0, 3, 15, and 75 ppm, produced minimal maternal toxicity at the high-dose level. Mortality, food/water consumption, and body weight were not affected in either generation, but there was a decrease in body-weight gain observed in the F0 females. There was no effect demonstrated on pregnancy rate, gestation times, or the ability to rear young to weaning, and the pre-coital time was comparable among the groups in both generations. F0 males displayed increased heart weight at the mid- and high-dose levels (dose-related) and increased liver and kidney weights at the high-dose level. F0 females displayed increased brain and liver weights at the high-dose level. In the F1b adults, the high-dose males displayed increased kidney weights compared to the controls and females displayed increased liver weights at the mid- and high-dose levels. There was no effect of treatment on litter size throughout both matings of both generations. In the first mating of the F0 generation, there was an increase in the cumulative litter loss (%) at the high-dose level. Litter and pup weights were comparable at birth among the groups in both generations, but there was a decrease in litter weight observed in both matings in the F0 generation, which was significant at the high-dose in the first mating and at the mid- and high-dose levels in the second mating (dose-

related). Because there was no corroborative finding of a decrease in the number of pups per litter or in pup weight, the decrease in litter weight is not considered to be treatment-related. Increased pituitary weights (high-dose ♀♀ pups of 1st mate in F0 generation) and increased uterine weights (high-dose ♀♀ pups of 1st mate of F1b generation) were observed in the offspring. There were no histopathological findings observed that could be attributed to treatment.

The NOEL for maternal toxicity can be set at 3 ppm (≈ 0.2 mg/kg/day), the LEL at 15 ppm (≈ 1.0 mg/kg/day), based on increased liver weight. The NOEL for effects on the offspring can be set at 15 ppm, the LEL at 75 ppm, based on increased pituitary and uterine weights. A NOEL for reproductive effects can be set at 75 ppm (≈ 5.0 mg/kg), the highest dose tested. Although there were no significant effects noted on the dams, the dose levels are considered adequate, based on the results of the range-finding study in which there was an increase in cumulative pup loss and a reduction in litter size at the 100 ppm dose level at days 24 and 28 post weaning. This study is remains classified Core minimum, and it satisfies the guideline requirements (83-4) for a 2-generation reproduction study.

NOTE: As recommended by the RfD Committee (memo dated May 15, 1992), this reproduction study in rats has been reevaluated. The effects observed at the mid-dose level are listed below.

MID-Dose ADULTS - A dose-related (1) increase in heart weight in F0 males; (2) increase in liver weight F1b females; (3) decrease in litter weight in the F0 generation, 2nd mating. Since the latter finding was not accompanied by a comparable decrease in pup weight or in the number of pups per litter, it is not considered to be treatment-related.