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

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JUN 26 1992

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Endosulfan: Re-evaluation of 2-Generation Reproduction Study in Rats

TO: George Z. Ghali, Ph.D.
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 6/22/92
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel JUN 23 1992
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia Gemert 6/23/92
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Action Requested: Re-evaluate the two-generation reproduction study in rats on Endosulfan and determine whether the effects observed at the middle dose level are treatment-related.

Comment: As per the recommendation of the RfD/Peer Review Report of Endosulfan (dated May 15, 1992), the 2-generation reproduction study in rats has been re-evaluated, and the DER is attached. 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

1

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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. 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 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 classification of this study is not altered by this re-evaluation; it remains: Core: Minimum. This study satisfies the guideline requirements (83-4) for a 2-generation reproduction study.

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)

JUN 23 1992

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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-hexahydr[illegible]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

3

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: Crl: COBS CD®(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 intersparsed 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. 7; 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 (≈ 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 ≈ 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 ≈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.

Achieved Intakes of Endosulfan (mg/kg/day)

Week / Group*	MALES 3	MALES 15	MALES 75	FEMALES 3	FEMALES 15	FEMALES 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	7.44
4	0.22	1.13	5.61	0.28	1.43	7.32
5	0.20	0.99	4.99	0.24	1.21	6.32
6	0.18	0.94	4.69	0.23	1.23	6.62
7	0.18	0.87	4.43	0.22	1.12	5.75
8	0.16	0.87	4.12	0.21	1.07	5.44
9	0.16	0.76	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
Over 12	0.20	1.00	4.99	0.24	1.25	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	9.85
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	6.54
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
Over 18	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) Low	Mid	High	Females (% of Control) Low	Mid	High
0	101	101	101	100	100	100
1a	103	101	101	100	99	96
2	103	102	102	99	98	96
3	104	102	102	100	99	96
4	105	103	103	100	100	98
12a	104	101	102	100	99	99
15	103	103	101	98	96	96
16	104	101	102	98	96	97
17	105	101	103	99	97	98
21a	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 0-1a	107*	104	102	100	96	87***
BWG 0-4a	108**	105	102	100	96	91*
BWG 0-12a	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	94	93

* p<0.05; ** p<0.01; *** p<0.001; a start of pre-mating period; a start of mating period; BWG - body-weight gain; BWGa first pre-mating period; (-) not calculated for males; NOTE: statistics not performed on BGW 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 ≥ 90% of the control value. There were no differences noted in body-weight gain in either sex.

F1b Generation Group Mean Body Weights (% of Control)

Week	Males (% of Control) Low	Mid	High	Females (% of Control) Low	Mid	High
4*	94	91	89	88	93	93
9	98	96	92	94	94	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) Low	Mid	High	F1b Body Weight Change (% of Control) Low	Mid	High
FIRST MATE 0-7	85	92	77	118	114	110
7-14	91	94	88	108	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	118	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.

10

(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.

12

Macroscopic Findings in Adult Males at Necropsy

Macroscopic Finding/ Generation/Dose group*	F0 Generation				F1b Generation			
	0	3	15	75	0	3	15	75
kidneys - males N=	32	32	32	32	28	28	28	28
enlarged	3	3	3	7	0	0	0	0
liver - males N=	32	32	32	32	28	28	28	28
enlarged	4	5	5	10	0	1	0	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
kidneys - females N=	32	32	32	32	28	28	28	28
enlarged	0	0	0	0	0	0	0	0
liver - females N=	32	32	32	32	28	28	28	28
enlarged	0	0	0	2	0	0	0	0
median cleft accessory lobe	nr	nr	nr	nr	0	0	0	2
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 (96)
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 $\left(\frac{\text{(\# of pups born - \# of pups surviving)}}{\text{\# of pups born}} \times 100\right)$

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.

F0 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 weights	67.4	72.3	72.6	68.4
pup weights	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	4.7	7.8

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

F0 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	12.4/13.0	12.3/12.6	12.8
% 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	6.3/6.0	6.0/6.3	5.9/6.4	5.9/5.6
LITTER SIZE				
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
LITTER WEIGHT				
Day 4	121.0	115.8	113.0*	112.1
Day 8	191.2	185.6	177.6*	172.5*
Day 12	472.7	264.8	252.8**	240.8**
Day 21	491.3	485.5	468.6	443.8
MEAN PUP WEIGHT				
Day 4	9.8	9.4	9.2	9.7
Day 8	15.7	15.3	14.7	15.8
Day 12	22.4	22.1	20.8	21.2
Day 21	40.3	40.5	38.6	39.6
CUMULATIVE LOSS (%)				
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 ♂ values or as Mean ♂/Mean ♀ 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	6.8/6.5	5.3/6.3
sex ratio ♂/♀ day 21	5.3/5.9	5.4/5.4	5.8/5.5	4.8/5.4
LITTER SIZE				
Day 4	11.6	11.6/11.6	12.8/12.7	11.0/11.2
Day 8	11.6	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
LITTER WEIGHT				
Day 4	82.8	89.6	90.5*	82.8
Day 8	129.2	141.3*	134.8	124.8
Day 12	186.5	205.4	200.1	177.7
Day 21	355.9	405.6	376.9	339.9
MEAN PUP WEIGHT				
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	8.8/8.6	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 loss♦	1.7/1.7	1.5/1.2	0	1.1
Litter weight♦	67.6	64.2	73.5	68.3
pup weight♦	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	6.2/5.6	5.6/5.5	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	10.9/10.9	13.4	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 (111)	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	96

• ( ) 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.