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004881

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of the following studies submitted as follow-up to the 1982 Endosulfan Registration Standard

1. Preliminary Investigation of the Effect of Endosulfan on Reproduction of the Rat; Edwards, J.A., Hughes, E.W., Almond, R.H., Huntingdon Research Centre, Huntingdon, Cambridgeshire, England for Hoechst Aktiengesellschaft, Frankfurt, W. Germany; December 2, 1982; A 29563, HST 203/82253; EPA Accession No. 256126.

2. Effect of Endosulfan- Technical on the Reproductive Function of Multiple Generations in the Rat; Edwards, J.A., et. al.; Huntingdon Research Centre, Huntingdon, Cambridgeshire, England tor Hoechst Aktiengesellschaft, Frankfurt, W. Germany; July 19, 1984; A 29428, HST 204/83768, EPA Accession No. 256127.

3. Addendum to HST 204 Effect of Endosulfan- Technical on the Reproductive Function of Multiple Generations in the Rat; Histopathological review of the kidneys in adult rats of the F_{1B} generation and in weanling rats of the F_{2B} generation; Offer, J.M., Huntingdon Research Center plc, Huntingdon, Cambridgeshire, England; March 22, 1985; A 30757; Addendum to HST 204/83768, EPA Accession No. 257727.

TO: George Larocca, Product Manager 15
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FROM: Margaret L. Jones Margaret L. Jones 1/3/86
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THROUGH: Clint Skinner, PH.D., Head Clint Skinner 1-8-86
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and Theodore M. Farber, Ph.D., D.A.B.T., Chief
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Compound: Endosulfan Technical Tox. Chem: 420

1 of 29

SEC RD #

Registration No.: 154116, 154117, 154118 Registrant: American Hoechst

Accession No.: 256126, 256127, 257727

Tox. Project Nos.: 86, 492

Action Requested: Review of the preliminary reproduction study, the multiple generation reproduction study, and the histopathology follow-up to the two-generation reproduction study, which were IBT replacement studies, cited as "data gaps" in the 1982 Endosulfan Registration Standard.

Background: A preliminary study was performed to determine the doses to test in the multiple generation study. The conclusions of this study are cited below. The multiple generation reproduction study was done and a follow-up histopathology investigation was done to check for certain kidney effects which were originally seen in the 30 day and 13 week rat feeding studies.

Conclusions in Preliminary Investigation: The most significant effects on reproduction of Endosulfan Technical were the progressive loss of individuals after birth and lowered litter weights from birth to day 28 at all doses. This study did not demonstrate a no observed effects level (NOEL) for increased liver weights in females. The NOEL for lowered body weight in dams was 50 ppm. The NOEL for lowered litter weight and for increased litter loss was 50 ppm. The study was classified as Supplementary.

Conclusions in Multiple Generation Study and Histopathology Addendum: Endosulfan Technical exposure at dietary levels of 0, 3, 15, and 75 ppm produced increased numbers of females without viable young in F_0 and F_{1B} females at both matings. This effect did not appear at 75 ppm in the F_0 generation and there was a lowered incidence in the F_{1B} generation than in the F_0 generation. The NOEL for lowered mean litter weight was 3 ppm.

The NOEL for organ weight effects in adults was 3 ppm, the effect was increased mean heart weight. Heart weight was significantly increased at 15 and 75 ppm in the F_0 males only. This effect was not seen in the F_0 females, in either sex of the F_{1B} adults, or in the weanlings. In addition, increased heart weight was not seen in the 90 day oral rat study which used doses of 0, 10, 30, 60, and 360 ppm. Since this effect was seen only in one sex in one generation it is most likely a spurious effect.

There was no NOEL for the observed yellowish discoloration of the cells of the proximal convoluted tubules of the kidney, which was found in traces in F_{1B} males at all doses, and in minimal amounts in males at 75 ppm only (See Attachment 10). The yellowish discoloration and traces of granular clumped pigment observed in the cells of the proximal convoluted tubule of the kidney

are indicative of some destruction of red blood cells in the hematopoietic system, which apparently occurs at all doses tested in males. The studies were classified as Minimum.

004881

DATA EVALUATION REPORT

Chemical: Endosulfan Technical; Tniodan®; 6,7,8,9,10,10- Hexachloro -1,5,5a, 6,9,9a -hexahydro -6,9 -methano -2,4,3 benzodioxathiepin -3- oxide.

Citation: Preliminary Investigation of the Effect of Endosulfan (Code HOE 02671 OI AT 209) on Reproduction of the Rat; Edwards, J.A., Hughes, E.W., Almond, R.H., Huntingdon Research Centre, Huntingdon, Cambridgeshire, England for Hoechst Aktiengesellschaft, Frankfurt, W. Germany; December 2, 1982; A 29563, HST 203/82253, Acc. No. 256126 Caswell No. 420

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Core Classification: Supplementary (range finding study)

Conclusions: The most significant effects on reproduction of Endosulfan Technical were the progressive loss of individuals after birth and lowered litter weights from birth to day 28 at all doses. This study did not demonstrate a no observed effects level (NOEL) for increased liver weights in females. The NOEL for lowered body weight in dams was 50 ppm. The NOEL for lowered litter weight and for increased litter loss was 50 ppm.

Materials

Test Material: Endosulfan Technical, Batch No. HOE 02671 OI AT 209; Certificate of analysis no. 01616 (April 30, 1981). 97% pure

Test Animals: Eighty male and female rats of Crl:COBSCD (SD)BR strain from Charles River (UK), Margate, Kent were allowed to acclimate for 7 days and were then distributed randomly to test groups. They were then allowed an additional 7 day acclimation period prior to testing.

Test Methods: Four groups of 10 male and 10 female rats were dosed with 0, 50, 75, and 100 ppm Endosulfan in the diet. The test preparation was analysed to verify homogeneity and level of test substance in the feed.

Handling of Animals: Males and females were housed 5 per cage with cages holding the males placed between the cages holding

females to promote regular oestrus cycles. One male and one female were housed in plastic breeding cages during the mating period. After mating, males were returned to their original group cages and females remained in the breeding cages. Dams were returned to their original group cages after weaning the litters (day 21 postpartum). The pups remained in the breeding cages for one additional week and members of litters of 7 or more were dispersed among two cages. F_0 animals were maintained on the diet containing Endosulfan from a date two weeks prior to mating until sacrifice, after weaning of offspring.

Observations:

Parental animals were observed for signs of toxicity during the study. Food consumption was measured weekly and the food conversion ratio was calculated using this data and body weight data, which was measured weekly. Intake of test material was calculated from the dietary concentration, weekly food consumption, and mean mid-week body weight, as follows:

$$\text{Intake of test material (mg/kg/day)} = \frac{\text{Dietary conc. (ppm)}}{10^6} \times \frac{\text{Mean food conc. during week (g/rat/week)}}{\text{mean mid-week body weight (kg)}} \times \frac{1000}{7}$$

The dams were weighed on days 0,7,14,17, and 20 of gestation, after evidence of mating was found (sperm or plug). After litters were delivered, dams were weighed on postpartum days 0,7,14,and 21. Pregnancy rate was the percentage of surviving paired females that became pregnant. Mating performance was assessed from vaginal smears taken during the mating period to determine whether or not pregnancy was interrupted after mating, whether there were any anomalies of the oestrus cycles, and the length of the mean pre-coital time for the group. Gestation was the time between successful mating and parturition. The parental animals were killed by CO_2 asphyxiation, then examined macroscopically and the liver weighed. The uterus of one non-pregnant female was examined (Salewski technique) to detect early implantation losses, if any. Testes, prostate, and seminal vesicles of the male who failed to impregnate were weighed and preserved in 10% buffered formalin.

Litters were examined by first identifying members of each litter, sexing and weighing each, and examining them for external abnormalities. Pups were weighed on days 4,8,12,and 21. Weaning occurred on day 21 and weights were taken on days 24 and 28 (postpartum). Seven days after weaning, the pups were asphyxiated and examined externally and internally for any abnormalities. Those with possible skeletal abnormalities were x-rayed.

Statistical analysis was performed, using the non-parametric tests of Jonckheere and Kruskal-Wallis for litter data, which did not follow a normal distribution, and Analysis of covariance and Williams' test to analyse liver weights.

Results:

No toxic signs were observed following treatment. One female at 75 ppm was sacrificed after parturition due to weight loss, hunched posture and piloerection. Mating performance and pregnancy rate were not affected by treatment. Duration of gestation was normal as compared to controls.

One male at 75 ppm failed to mate. At 75 ppm one female was not pregnant and one was sacrificed after giving birth. In the female control group, one experienced total litter loss.

Food conversion ratios (see Table I) were increased at 75 and 100 ppm in females during week 1 and lowered during week 2, as compared to controls.

Group mean food consumption was lowered in females at 50, 75, and 100 ppm during the first week of treatment. There was apparently no difference after the first week.

Group mean body weight was not significantly different in treated males and controls. In treated females, mean body weights were lowered at 75 and 100 ppm. Following parturition, group mean body weights of the dams were similar in all dose groups (0, 50, 75, and 100) until terminal sacrifice. Table II shows the weights of interest.

Endosulfan Technical produced several litter effects. These are shown in the attached photocopies of Tables 10A and 10B from Test Report HST 203/82253. Starting with day 4 there was an increased cumulative loss of individuals in litters at all doses compared to controls. The loss was progressive over the 28 day observation period from birth to one week following weaning at day 21 and generally compound-related. The percent loss was as great as 5 times the control value at 100 ppm by days 24 and 28. Litter weights were lower than controls in all dose groups and the weight losses were dose related. At 75 and 100 ppm the differences were statistically significant for decreased litter weight when compared to controls. At 100 ppm on days 24 and 28 the differences in litter sizes were statistically significant. The differences at 75 and at 50 ppm were biologically significant.

Sex ratios at weaning generally reflected the ratios at birth. The ratios of females to males were smaller at 75 and 100 ppm as compared to controls.

Mean liver weights were significantly elevated in females at 50, 75, and 100 ppm as compared to controls. Male liver weight was not affected. These values are recorded in Table III.

No gross macroscopic effects were noted in the F_0 group at autopsy other than the increased liver weights described above. Likewise, in pups, no gross macroscopic effects were noted at autopsy.

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Table I. Food Conversion Ratios
(g food consumed/ g weight gain) in Weeks 1 and 2**

Week/ppm	0	50	75	100	0	50	75	100
1	4.5	4.4	4.3	4.0	9.9	7.9	19.2	a
2	6.1	6.0	5.6	5.5	15.8	15.9	12.0	10.9

** From Table 5 of Test Report HST 203/82253, p. 20.
a. No weight gain occurred.

Table II. Group Mean Weekly Body Weights (g)*

Week of Study	Gestation Day (approx)	Females 0	75	100	ppm
-1	(Acclimation	193	192	192	191
2	& Mating)	223	225	211	205
3	0	234	233	224	224
4	(7 Gestation	268	263	250	252
5	14	302	298	286	287
6	20	364	364	345	344
7	Postpartum Day 0/1	284	273	268	269
8	7	279	290	274	275
9	14	302	302	283	291
11	21	290	286	269	281

* From Table 4 of Test Report HST 203/82253, p. 19.

Table III. Mean Adult Liver Weights (g)

	Males	Females
0	26.1	12.4
50	26.9	14.8[4]
75	24.0	14.0[3]
100	26.9	15.0[4]

3. Significant at $p<0.05$ as compared to controls.
4. Significant at $p<0.01$ as compared to controls.

Attachment 1

TABLE 10A

Group mean litter data from birth to weaning

Group:	1	2	3	4
Compound:	Control		Endosulfan	
Dietary concentrations (ppm):	-	50	75	100

Group	No. of animals		At birth					At day 4				At day 8				At day 12				At day 21					
	Mated	Pregnant #	Litter size			Litter wt. (g)	Mean pup wt. (g)	Litter size	Cumulative loss %	Litter wt. (g)	Mean pup wt. (g)	Litter size	Cumulative loss %	Litter wt. (g)	Mean pup wt. (g)	Litter size	Cumulative loss %	Litter wt. (g)	Mean pup wt. (g)	Litter size			Cumulative loss %	Litter wt. (g)	Mean pup wt. (g)
			Total	Live	Loss %															♂	♀	Total			
1	10	A = 10	13.7	13.5	1.5	-	-	13.4	2.2	-	-	13.1	4.3	-	-	11.9	14.3	-	-	-	-	11.9	14.3	-	-
		B = 9	13.9	13.7	1.7	76.2	5.6	13.6	2.4	114.4	8.5	13.2	4.8	172.8	13.2	13.2	4.8	260.5	19.9	6.2	7.0	13.2	4.8	474.1	36.3
2	10	A=B=10	14.7	14.3	2.3	80.5	5.7	13.8	5.5	110.7	8.0	13.4	8.0	165.5	12.3	13.1	9.8	240.7	18.2	6.1	7.0	13.1	9.8	436.0	32.9
3	10	A=B= 8	13.6	13.5	0.8	76.8	5.7	12.9	5.4	97.4+	7.6	12.4	8.7	141.1+	11.5	12.1	10.3	208.7+	17.3	6.3	5.8	12.0	11.1	362.5+	30.4
4	10	A=B=10	12.4	12.3	0.6	71.0	5.8	11.6	8.3	87.2++	7.6	11.3	10.4	128.2+	11.4	11.0	12.6	184.9++	16.6+	6.0	4.6	10.6	15.7	320.1+	30.3
Kruskal-Wallis 'H' statistic mean B values			NS	NS	NS	NS	NS	NS	NS	P<0.01	NS	NS	NS	NS	NS	NS	NS	P<0.05	NS	-	-	NS	NS	P<0.05	NS
Jonckheere 'J' statistic mean B values			NS	NS	NS	NS	NS	NS	NS	P<0.001	NS	NS	NS	P<0.05	NS	NS	NS	P<0.01	P<0.05	-	-	P<0.05	NS	P<0.01	NS

Intergroup comparisons with controls based on Kruskal-Wallis mean ranks: + P<0.05
++ P<0.01

NS Not significant P>0.05

For definition of mean A and mean B values see page 6

A ≡ Mean of data from surviving animals with evidence of pregnancy including those subsequently losing the entire litter, ie. failing to wean any young

B ≡ mean of data from any animals with young surviving to weaning

Attachment 2

TABLE 10B

Group mean litter data following weaning

Group:	1	2	3	4
Compound:	Control		Endosulfan	
Dietary concentration (ppm):	-	50	75	100

Group	No. of animals		At Day 24				At Day 28			
	Mated	Pregnant	Litter size	Cumulative loss %	Litter wt (g)	Mean pup wt (g)	Litter size	Cumulative loss %	Litter wt (g)	Mean pup wt (g)
1	10	A=10	11.9	14.3	-	-	11.9	14.3	-	-
		B= 9	13.2	4.8	623.6	47.7	13.2	4.8	832.9	63.6
2	10	A=B=10	12.1	16.2	520.6	41.4	11.7	18.4	710.9	58.2
3	10	A=B= 8	12.0	11.1	488.6+	41.0	12.0	11.1	686.3	57.5
4	10	A=B=10	9.2+	25.8(+)	382.3++	39.5	9.1+	26.5(+)	529.4++	55.8
Kruskal-Wallis 'H' statistic mean B values			NS	NS	P<0.05	NS	NS	NS	P<0.05	NS
Jonckheere 'J' statistic mean B values			P<0.05	NS	P<0.01	NS	P<0.05	NS	P<0.01	NS

Intergroup comparisons with controls based on Kruskal-Wallis mean ranks: + P<0.05
++ P<0.01

() Not supported by significant 'H' or 'J' statistic
NS Not significant P>0.05

DATA EVALUATION REPORT 004881

Chemical: Endosulfan Technical; Thiodan®; 6,7,8,9,10,10- Hexachloro -1,5,5a, 6,9,9a -hexahydro -6,9 -methano -2,4,3 benzodioxathiepin -3- oxide.

Citation: Effect of Endosulfan - Technical (Code:HOE 02671 OI AT 209) on Reproductive Function of Multiple Generations in the Rat; Edwards, J.A., et. al.; Huntingdon Research Centre, Huntingdon, Cambridgeshire, England for Hoechst Aktiengesellschaft, Frankfurt, W. Germany; July 19, 1984; A 29428, HST 204/83768, Acc. No. 256127 Caswell No. 420

and Addendum to HST 204 Effect of Endosulfan - Technical (Code: HOE 02671 OI AT 209) on the Reproductive Function of Multiple Generations in the Rat; Histopathological review of the kidneys in adult rats of the F_{1B} generation and in weanling rats of the F_{2B} generation; Offer, John, M., Huntingdon Research Center plc, Huntingdon, Cambridgeshire, England; March 22, 1985; A 30757; Addendum to HST 204/83768; Acc.No. 257727; Caswell No. 420

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Core Classification: Minimum

Conclusions:

Endosulfan Technical exposure at dietary levels of 0, 3, 15, and 75 ppm produced increased numbers of females without viable young in F_0 and F_{1B} females at both mates. This effect did not appear at 75 ppm in the F_0 generation and there was a lowered incidence in the F_{1B} generation than in the F_0 generation. The NOEL for lowered mean litter weight was 3 ppm.

The NOEL for organ weight effects in adults was 3 ppm, the effect was increased mean heart weight. Heart weight was significantly increased at 15 and 75 ppm in the F_0 males only. This effect was not seen in the F_0 females, in either sex of the F_{1B} adults, or in the weanlings. Since this effect was seen only in one sex in one generation it is most likely a spurious effect.

There was no NOEL for the observed yellowish discoloration of the cells of the proximal convoluted tubules of the kidney, which was found in traces in F_{1B} males at all doses, and in minimal amounts in males at 75 ppm only (See Attachment 10). The above observation and traces of granular clumped pigment seen in the cells of the proximal convoluted tubule of the kidney are indicative of some destruction of red blood cells in the hematopoietic system, which apparently occurs at all doses tested in males.

Materials

Test Material: Technical grade Endosulfan (HOE 02671 OI AT 209); Certificate of Analysis No.: 01616 (30 April 1981); 97% pure. The test material was dissolved in acetone; corn oil was then added. This dispersant was mixed with the feed and then placed in a rotary evaporator to evaporate the acetone.

Test Animals: Four week old male and female rats of Crl: COBS CD (SD) BR strain from Charles River UK, Ltd. were allowed to acclimate for 7 days ; and were then distributed randomly to groups of 32 of each sex for the F_0 generation. F_{1B} animals were distributed randomly to groups of 28 of each sex for the second generation.

Study Design

1. The attached Figure i (Attachment 1) from the test report shows the study design. The following table shows the numbers of animals tested at each dose level and generation.

		Two Generation Reproduction			
Test Group	Dose in Diet (ppm)	F_0 males	females	F_{1B} males	females
1	0	32	32	28	28
2	3	32	32	28	28
3	15	32	32	28	28
4	75	32	32	28	28

During pre-mating and between matings, the animals were housed four to a cage. The rats were fed 0, 3, 15, and 75 ppm starting at 6 weeks of age. While mating, one female and one male were caged together. After mating the females remained in separate cages through birth and weaning. Mating commenced when the animals were 18 weeks of age.

2. Feed: Spratt's Laboratory Diet No. 2

Water: Tap water

Water and feed were analysed to ensure proper levels of major nutrients, vitamins, and minerals were present and contaminants kept to a minimum. (Data stored at HRC Archives.) Diets were prepared fresh each 2 weeks. Diet for the first week was kept in the animal room and the feed for the 2nd week was kept at 4° C.

3. The following statistical methods were used to determine the significance of the results:

Authors/Method	Data Analysed
Kruskal-Wallis[1]. and Jonckheere[2].	Litter data
Analysis of Variance William's[3].	Organ weights Intergroup comparisons 1.

1. Kruskal, W.H. and Wallis, W.A. (1952/3). J. Amer. Statist. Ass. 47: 583-621 and 48: 907-912
2. No reference given in Test Report
3. Williams, D.A. (1971/2). Biometrics 27: 103-117 and 28: 519-531

Methods

1. Adult Performance:

F_0 and F_{1B} generations were mated twice as shown in the study design in Attachment 1. Sacrifice of F_{1A} pups took place at 21 days. One male and one female of each litter were preserved for histopathological examination. F_0 animals were remated to different mates after weaning the F_{1A} litters. The young were reared until day 21 post partum, when 28 male and 28 female pups from each group were selected to form the F_{1B} generation which was mated twice, taking care not to mate siblings.

2. Body Weight:

Body weight was recorded before placing animals on test diet and at weekly intervals thereafter. During mating, females were weighed on alternate days. When pregnancy was confirmed the dams were then weighed on days 0, 7, 14, and 20 of gestation. After delivery of litters, dams were weighed on days 0, 7, 14, and 21 post partum.

3. Litter Data:

Young were counted, marked for identification, sexed, weighed, and examined for external abnormalities. Examination for any dead and abnormal young took place each day and pup weights were recorded on days 4, 8, 12, and 21. Mean values were calculated for total number of young, number of live young per litter, litter weight from individual pup weights, and percent of litter loss at birt and on days 4, 8, 12, and 21 post partum.

4. Organ Weights:

One male and one female pup per litter and all F_{1B} adults were selected for organ weight analysis. Pups were first examined internally and externally for macroscopic abnormalities then selected organs were weighed. Adult organ weights were measured

after the second weaning. The following adult organs were weighed:

liver	adrenals	pituitary
kidneys	spleen	testes and epididymides/or
heart	brain	ovaries and uterus

For males failing to impregnate at the second mating the prostate and seminal vesicles were weighed. To find evidence of implantation in females without viable young at the second mate, the uteri were placed in 10% ammonium sulphide solution. Around day 21 postpartum one female and one male pup per litter were sacrificed for organ weight analysis.

5. Microscopic Examination of Tissues

Histopathology examination of F_{1B} adults included the tissues indicated on the following page with an "*" and was limited to controls and the 75 ppm group and males failing to induce pregnancy at the second mate from all dose groups had testes, prostate, and seminal vesicles examined. The uterus of females without viable young at the second mate were preserved and examined. When the organs of selected pups were preserved for analysis, the tissues showing any abnormality were also preserved for histopathology examination. Histopathology examination was limited to selected pups from the F_{1B} second mate: litters from the control and the 75 ppm dose groups.

Samples of all the following tissues were preserved:

adrenals*	mammary gland*	urinary bladder*
aorta	salivary gland	uterus*
bone marrow*	sciatic nerve	vagina*
brain*	second eye	mid-colon*
caecum	seminal vesicles*	oesophagus
duodenum	skeletal muscle	ovaries*
epididymides*	skin*	pancreas*
eye*	spinal cord	pituitary*
heart*	spleen*	prostate*
ileum*	stomach*	
jejunum	testes*	
kidneys*	thymus*	
liver*	thyroids*	
lungs*	tongue	
lymph nodes*	trachea	

6. Kidney Histopathology: Addendum to HST 204: Histopathological review of the kidneys in adult rats of the F_{1B} generation and in weanling rats of the F_{2B} generation; Discussion with Toxicology Branch Pathologist, Dr. L. Kasza, D.V.M.

Background: In a 13 week toxicity study in rats followed by a 4 week withdrawal period using Endosulfan, yellowish discoloration and pigment deposits in renal proximal tubules were found. [Citation: Endosulfan-Active Ingredient Technical (Code: HOE 002671 OI ZD 97 0003) 13 week toxicity study in rats followed by a 4-week withdrawal period (Final Report) Barnard, A.V., et.al., Huntingdon Research Center, Huntingdon, Cambridgeshire, England for Hoechst Aktiengesellschaft, Frankfurt, W.Germany; March 25, 1985; A 30700, HST 230/84176; Acc. No. 257727]. For this reason, a follow-up histopathology

examination of the kidneys from animals from the multigeneration reproduction study was undertaken. Kidneys from twenty eight F_{1B} rats of each sex from 0, 3, 15, and 75 ppm dose groups were reexamined. F_{2B} generation weanling rats from the 0 and 75 ppm dose groups were examined.

Results

1. Adult Performance:

Tables 2A and 2B (See Attachments 2 and 3) from Report No. A 29428 summarize adult performance in the F_0 and F_{1B} generations. (1) There were three mortalities in females of F_0 at 0, 3, and 15 ppm. (2) In the F_0 at both mates there were increases over controls in the numbers of females without viable young at 3 and 15 ppm. At 75 ppm the effect appeared at control level. In the F_{1B} generation there was a compound-related increase over controls in the number of females without viable young at both mates. (3) Total litter loss was seen in the F_0 generation at the second mate in one female at 0, 3, and 15 ppm. Total litter loss was seen in the F_{1B} generation in one female at 3, 15, and 75 ppm in the first mate, and in one female at 0, and 3 ppm in the second mate. These losses do not appear to be significant.

2. Body Weight:

Group mean body weights were lower in the F_0 generation than controls at 15 and 75 ppm during the second mating, gestation, and post partum periods. Body weights in these groups were similar to control values by day 21 post partum, when the F_{1B} offspring were were weaned. Analysis of body weight gains showed statistically significant differences between F_{1B} females and controls at 3 ppm through most of the observation period. Other statistically significant differences were seen in F_0 males at 3 ppm during weeks 0-16 and in F_0 females at 75 ppm for weeks 1-4.

3. Litter Data:

Tables 12A, 12B, 12C, and 12D from Test Report No. A 29428 show these results. (See Attachments 4-11.)

F_0 generation: In litters from the first mate, there was increased cumulative percent loss at 75 ppm as compared to controls and lowered mean litter weight was seen at 75 ppm from day 4 through weaning (day 21). The mean litter weight in pups was significantly lower than controls at $p<0.05$ (Jonckheere) on days 8, 12, and 21.

At the second mating there was an increased cumulative percent litter loss over controls at birth in the 75 ppm group. This effect disappeared by day 8. Lowered mean litter weights were seen at 15 and 75 ppm from day 4 through 21. The loss was significant at $p<0.05$ (Jonckheere) on days 4 and 21 and at $p<0.01$ (Jonckheere) on days 8 and 12 post partum.

F_{1B} generation: Litter losses and lowered litter weight were not consistently seen in the two matings of this generation. Slightly lowered mean litter weights were seen in the second mating

at 75 ppm on days 12 and 21 post partum. At 3 ppm, in the first mate of the F_{1B} generation, litter weight and mean pup weight were significantly ($p<0.05$) greater than control values from day 4 through 21.

4. Organ Weights:

Adult Organ Weights

F_0 generation: In males, increased mean heart weights were found at 3, 15, and 75 ppm. These differences were significant at 15 ppm ($p<0.05$) and at 75 ppm ($p<0.01$) when values were adjusted for bodyweight as a covariate (Williams' test). Increased mean kidney weights were found at 75 ppm and were significantly ($p<0.01$) different from controls when adjusted for bodyweight as covariate. When mean liver weights at 75 ppm were adjusted similarly they were found to be significantly increased at 75 ppm ($p<0.05$).

In females, at 75 ppm, mean brain weight ($p<0.05$) and mean liver weight ($p<0.01$) were significantly increased over controls when adjusted as above.

F_{1B} generation: In males, slightly increased mean liver weight at 75 ppm, and significantly increased ($p<0.01$) mean kidney weight were found at 75 ppm ($p<0.01$). In females, increased mean liver weights were found at 15 ($p<0.01$) and 75 ppm ($p<0.001$), and slightly elevated mean kidney weights were found at 75 ppm, as compared to controls.

Weanling Organ Weights

There were no statistically significant differences between control and treated groups in mean organ weights of weanlings in either mate of the F_0 or F_{1B} generations.

5. Microscopic Examination of Tissues:

F_{1B} adult rats from the control group and the 75 ppm dose group were examined microscopically. F_{2B} weanling rats from the control group and the 75 ppm dose group were also examined microscopically. The significant findings are summarized in the following table.

Incidence and Distribution of Microscopic Findings in F_{1B} Adults [1]

	Males		Females	
	0	75	0	75
Liver				
Centrilobular hepatocyte) minimal	11	13	0	0
vacuolation) moderate	1	0	0	0
Fat deposition in) trace	8	8	0	0
centrilobular hepatocytes) moderate	5	2	0	0
Kidneys				
Early progressive glomerulonephrosis	5	6	0	2
Increased pelvic dilatation	0	0	2	2
Total Examined	28	28	28	28

Incidence and Distribution of Microscopic Findings in F_{2B} Weanlings [2]

	Males		Females	
Kidneys	0	75	0	75
Moderate increased pelvic dilatation	1	1	0	4
Total Examined	25	26	25	26

1. From Table 1, Addendum 2, p. 346 of Lab Report A 29428.
2. From Table 2, Addendum 2, p. 348 of Lab Report A 29428.

6. Histopathology Results and Discussion:

Table A from HST/204-Addendum (See Attachment 12) shows the incidence and distribution of renal changes in the F_{1B} adult rats at all doses. Yellowish discoloration of cells in the proximal convoluted tubules of the kidney were seen in males at 3, 15, and 75 ppm. In addition, granular clumped pigment was observed in trace amounts in these cells. No evidence of yellowish discoloration or granular pigment in the proximal convoluted tubule cells was noted in kidneys from weanlings from the F_{2B} generation.

Discussion with the Toxicology Branch pathologist revealed there are two possible explanations for the observed phenomena. One possible reason for such discoloration and pigment clumping is the administration of a pigmented compound, which could be deposited in these cells during passage through the kidney. A second possible explanation for the yellowish discoloration and pigment clumping found in cells of the proximal convoluted tubules of the kidney is an effect of the test substance on the hematopoietic system which involved some destruction of red blood cells.

Although the color of the test compound was not specified in Test Report 204/83768, another report which tested Endosulfan-Technical indicated the test compound consisted of "...buff-colored flakes...", (Test Report HST 230/84176, p.2, Certificate of Analysis No. 02184).

It seems unlikely the discoloration observed was due to the color of the test substance. The pigment found in the proximal convoluted tubule cells was most likely the by-product of the breakdown of red blood cells. This explanation agrees with observations in other studies with Endosulfan in which red blood cells and hemoglobin levels were depressed and in which dark-colored urine was observed (Test Report HST 230/84176, p.2, Certificate of Analysis No. 02184). (HST 230/84176, reference given on p. 4 of this review). Although hematology and biochemistry values were not measured in the two generation reproduction study, one would expect that hemoglobin levels and red blood cell counts would be slightly depressed in these animals, as was found in HST 230/84176.

In conclusion, Endosulfan appears to have an effect on the hematopoietic system, probably producing lower hemoglobin levels and RBC destruction, which will be more thoroughly examined in the reviews of the subchronic and 30-day toxicity studies in the rat.

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Endosulfan toxicology review

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