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

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OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCESMEMORANDUM

SUBJECT: Endosulfan Technical; Review of 13-Week Toxicity Study in Mice

TO: George LaRocca, Product Manager 15  
Registration Division (TS-767)

FROM: Margaret L. Jones  
Review Section IV  
Toxicology Branch  
HED (TS-769)

*Margaret L. Jones 10/21/85*

THROUGH: Robert P. Zendzian, Ph.D., Acting Head  
Review Section IV

*10/22/85*

and Theodore M. Farber, Ph.D., Chief  
Toxicology Branch

*10/23/85*

Compound: Endosulfan Technical

Tox. Chem: 420

Registration #: 154110

Registrant: American Hoechst

Accession #: 256114

Tox. Project No.: 493

Study Identification: Endosulfan - Active Ingredient  
Technical 13-Weeks Toxicity Study in Mice (Final Report),  
Barnard, A.V., et.al., Unpublished study conducted by  
Huntingdon Research Center plc, Huntingdon, Cambridgeshire,  
England for Hoechst Aktiengesellschaft, Pharma Forschung  
Toxicologie, Frankfurt, West Germany; 25 September 1984;

Action Requested: Review of the 13-week Toxicity Study in  
Mice with Endosulfan Technical which was identified as a  
"data gap" in the 1982 Endosulfan Registration Standard.

Background: The 13-week Toxicity Study in Mice with Endosulfan  
Technical was submitted to EPA after being required by the  
1982 Registration Standard. A previously reviewed subchronic  
study in rats showed effects at all doses tested and a no  
observed effects level could not be established. The  
requirement for a subchronic feeding study was therefore not  
fulfilled.

**Conclusions:** Increased mortality was produced by administration of 54 ppm Endosulfan Technical in the diet. 12/20 males and 10/20 females died at this dose. Glucose levels in females were significantly lowered at 54, 18, and 6 ppm. The no observed effects level (NOEL) for lowered glucose effects in females was 2 ppm. There was no NOEL for hematology effects: hemoglobin levels were significantly elevated at 2, 6, and 18 ppm in females and appeared elevated at 54 ppm, however this value was not analysed due to the few survivors (10/20) at this level, according to the test report. Mean corpuscular hemoglobin concentration was significantly lowered at 2, 6, and 18 ppm in females and appeared elevated at 54 ppm, however this value was not analysed for the reason cited above.

**Recommendations:** This study is designated core Minimum.

Chemical: Endosulfan; Thiodan® Technical

Test Material: Technical grade Endosulfan HOE 002671 OI ZD97-0003, (97.2% pure)

Study Identification: Endosulfan - Active Ingredient Technical 13-Weeks Toxicity Study in Mice (Final Report), Barnard, A.V., et.al., Unpublished study conducted by Huntingdon Research Center plc, Huntingdon, Cambridgeshire, England for Hoechst Aktiengesellschaft, Pharma Forschung Toxicologie, Frankfurt, West Germany, 25 September 1984; EPA Accession No.: 256114 (HST/229; A29663).

Reviewed By: Margaret L. Jones *Margaret L. Jones 10/23/85*  
Review Section IV

Approved By: *[Signature]* 10/25/85  
Robert P. Zendzian, Ph.D., Acting Head  
Review Section IV

Conclusions: Twelve males and ten females died after being fed 54 ppm Endosulfan Technical in the diet for 13 weeks. The no observed effects level (NOEL) for lowered body weight, for decrease in food efficiency, and for change in absolute and relative organ weights - lowered absolute heart, liver, and kidney weight, and elevated relative heart, liver, and kidney weight - in males was 18 ppm. Females showed no significant effect on body weight but showed lowered absolute and relative organ weight at 18 ppm for uterus and kidney. Therefore, the NOEL for organ weight effects in females was 6 ppm. The NOEL for lowered glucose levels in females was 2 ppm and there was no NOEL for hematology effects. The NOEL for histopathology was 18 ppm, the effect being vascular congestion and hemorrhage of the lungs.

Core Classification: Minimum

Quality Assurance: Q.A. Audit Statement No., HST 229/831052 was signed by K. W. G. Shillam on 6/8/84.

Materials:

Test Substance: Endosulfan Technical, 97.2% pure (HOE 002671 OI ZD97 0003), Certificate of Analysis No. 02184, 2/1/83.

Test Animals: 252 CD-1 (Caesarean-derived) mice (127 males, 125 females) from Charles River, Manston, Kent; 28 days old; body weight ranged from 7 g. (males) to 3 g. (females).

Methods: Five groups of 20 male and 20 female mice were given 0, 2, 6, 18, and 54 ppm Endosulfan Technical for 13 weeks in their diet. Control animals were given normal diet mixed with acetone and maize oil. Ten animals of each sex were sacrificed after an approximately 20 day observation period prior to the test period and examined macroscopically. Any abnormalities were further examined microscopically. Food and water were analysed to check for accuracy of amount administered and for contaminants.

Observations during the test included mortality and reactions to treatment. Body weight was recorded when animals were assigned to groups, one week prior to the start of treatment, and weekly until the end of the study. Food intake (g/mouse/week) was measured from the difference in the amount given and left by each cage of mice divided by the number of survivors for the majority of days in each week. Food Conversion Ratios were calculated from the above information using weight of food consumed per unit gain in body weight. These calculations were over 13 weeks of treatment. Mean intake of test material (mg/kg/day) was also calculated from the above information and using the information on how much test material was included in the diet. Hematology measurements were made in weeks 6 and 13 using blood taken from the orbital sinus of 10 male and 10 female mice from each group. Values measured were packed cell volume (PCV), hemoglobin (Hb), red cell count (RBC), reticulocyte count, total white cell count, differential count, and platelets. The following values were calculated from the above measurements:

mean corpuscular  
hemoglobin concentration (MCHC) =  $(\text{Hb} \times 100) / \text{PCV}$

mean cell volume (MCV) =  $(\text{PCV} \times 10) / \text{RBC}$

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Biochemistry values were measured in weeks 6, 12, and 13 from blood taken from the orbital sinus of 10 male and 10 female mice. Values measured in weeks 6 and 13 were glucose, total proteins, urea nitrogen, alkaline phosphatase, glutamic-pyruvic transaminase, glutamic oxaloacetic transaminase, gamma glutamyl transpeptidase, total bilirubin, and in week 12, total lipids.

At 13 weeks of treatment all survivors were killed by CO<sub>2</sub> asphyxiation, examined externally by observation and palpation and then internally to determine condition and appearance of the internal organs. Organ weights of adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, and uterus were taken. Eyes were preserved in Davidson's fixative, and remaining tissues were preserved in 10 % formalin. The following tissues were examined histopathologically:

adrenals	pituitary
aorta	prostate*
brain*	salivary gland
eyes	sciatic nerve
femur	seminal vesicle*
gastro-intestinal tract	skeletal muscle
gall bladder	skin
Harderian gland	spinal column*
head	spleen*
heart*	sternum
kidneys*	testes*
larynx & pharynx	thymus
liver*	thyroid*
lungs	trachea
lymph nodes	tongue
mammary gland	urinary bladder
ovaries*	uterus*
pancreas*	vagina

\* Tissues with this symbol were examined at dose levels less

Also, any abnormal-appearing tissue was prepared and examined. The complete selection from the above list was used to examine mice treated with 54 ppm and 18 ppm.

Statistical analysis was used to evaluate the differences between groups in food consumption and body weight. Student's "t" test was used for this analysis. Individual animal data was also analysed and values examined were body weight, clinical pathology, and organ weight.

Values which varied greatly (frequency of the mode was less than 75%) were analysed using Bartlett's test for heterogeneity of variance followed by Student's "t" test and William's test to find a dose-related response. Where frequency of the mode was 75% and greater Fisher's exact test and Mantel's test were used in evaluating statistical significance. Where mortality was high (group 5) statistical analysis was not performed. Data from groups with sufficient numbers of survivors was analysed (groups 1, 2, 3, 4).

Adherence to Good Laboratory Practices was ensured through compliance with GLP principles from OECD as set forth in 21 CFR Part 58, 1978.

## Results:

### Clinical Observations

One male and one female at 54 ppm Endosulfan experienced one episode of convulsions and salivation in week 5. The male was sacrificed while dying as a result of the episode and the female was found dead shortly after apparent recovery from the episode. Other clinical observations are discussed with the particular observation, as under food consumption and body weight data.

### Intake of Test Substance

The following chart shows the intake of test substance in mg/kg/day corresponding to ppm in the diet. The indicated amounts of substance in units of ppm were slightly higher than the actual amount ingested, as measured analytically.

Table I. Mean Intake of Test Substance

<u>Dose</u> (ppm)	<u>Males</u> (mg/kg/day)	<u>Females</u> (mg/kg/day)
2	0.24	0.27
6	0.74	0.80
18	2.13	2.39
54	7.30	7.52

Mortality

Mortality was increased by the administration of 54 ppm of Endosulfan. Deaths in the 54 ppm dose group occurred between weeks 2-12 with the majority of deaths occurring between 4 and 9 for males and 2 and 7 for females. There was a treatment-related effect on survival at 54 ppm.

Table II. Mortality (No. died/No. treated)

<u>Dose (ppm)</u>	<u>Males</u>	<u>Females</u>
0	0/20	0/20
2	1/20	0/20
6	2/20	0/20
18	0/20	0/20
54	12/20	10/20

Body Weight

Group mean body weight was lower in males at 54 ppm during the entire study, a biologically meaningful finding. Body weight was approximately 10% lower than controls for this group. Mean body weight gain in males was significantly lower than controls at 6, 18, and 54 ppm during week 1. The values were significant at  $p < 0.05$  at 6 and 18 ppm and  $p < 0.01$  at 54 ppm. During this time, food consumption was significantly lower than controls in males and females at 54 ppm. Females showed a group mean loss in body weight at this time, however, the difference was not statistically significant.

Table III. Group Mean Body Weight (g)

<u>Males</u>				
<u>Dose (ppm)</u>	<u>Week</u>	<u>0</u>	<u>6</u>	<u>13</u>
0		30	36	39
2		30	33	38
6		31	37	39
18		30	37	38
54		30	34	35
<u>Females</u>				
<u>Dose (ppm)</u>	<u>Week</u>	<u>0</u>	<u>6</u>	<u>13</u>
0		26	30	32
2		26	30	33
6		25	30	32
18		25	29	30
54		25	29	31

### Food Efficiency

Food Efficiency was decreased in males at 54 ppm as compared to controls. This reflected an increase in food consumption in males at this dose from week 3-13. The values listed in Table IV are from Test Report HST/229. Reviewer calculations are slightly different from these values. The exact method of calculating the food efficiency (ie. values used to calculate FCR) is not described in the test report.

The female control value is much greater than would be expected, possibly due to spillage of material. For the purposes of this review, one can consider the low dose to be essentially similar to controls. Considering this, females showed a decrease in efficiency with increasing dose as expected from male values and mortality figures.

Table IV. Food Consumption Ratio (FCR\*) For 1-13 Weeks

<u>Dose(ppm)</u>	<u>Males</u>	<u>Females</u>
0	45.2	67.4
2	46.3	51.2
6	47.1	51.2
18	51.1	69.7
54	62.7	60.9

\* FCR=  $\frac{\text{amount of food eaten (g)}}{\text{unit gain in body weight (g)}}$

### Organ Weights

Relative organ weights at 54 ppm were different from controls and from the other dose groups in the heart, liver (males only), spleen, kidney, and uterus (females only at 18 ppm). Mean absolute organ weights for males were lower than control weights for spleen at 54 ppm and for kidney at 18 ppm. Mean absolute liver weight in females was higher than control weight at 54 ppm and mean absolute uterus weight in females was lower than control weight at 18 ppm. The difference in absolute weight of spleen between controls and the 54 ppm male group was statistically significant, as indicated in Table V.

Table V. Relative Organ Weights (absolute weight) (g)

Females

Dose (ppm)	Heart	Spleen	Uterus	Kidney
0	0.0052 (0.16)	0.0041 (0.13)	0.0072 (0.22)	0.014 (0.45)
2	0.0051 (0.16)	0.0040 (0.12)	0.0062 (0.19)	0.014 (0.45)
6	0.0052 (0.16)	0.0043 (0.13)	0.0071 (0.21)	0.015 (0.44)
18	0.0053 (0.16)	0.0046*(0.14)	0.0051**(0.15)	0.014**(0.41)
54	0.0055*(0.17)	0.0045*(0.13)	0.0079 (0.24)	0.015 (0.44)

Males

Dose (ppm)	Heart	Liver	Spleen	Kidney
0	0.0057 (0.22)	0.053 (2.02)	0.0035 (0.13)	0.018 (0.67)
2	0.0057 (0.21)	0.054 (1.98)	0.0032 (0.12)	0.017 (0.65)
6	0.0058 (0.22)	0.055 (2.07)	0.0036 (0.14)	0.017 (0.66)
18	0.0056 (0.20)	0.055 (1.97)	0.0036 (0.13)	0.017 (0.60)
54	0.0062*(0.21)	0.056*(1.91)	0.0029*(0.10) <sub>1</sub>	0.019*(0.65)

\* These values show a relative increase in organ to body weight ratio.

\*\* These values show a relative decrease in organ to body weight ratio.

1. This value showed a statistically significant difference between controls and dose group at  $p < 0.05$  (William's Test).

Hematology

Table VI. Group Mean Hematology Values

Males

Dose (ppm)	Neutrophils X $10^3$ /cmm		MCHC(%)	Hb(g/dl)
	wk 6	wk 13		
0	1.35	1.28	No significant differences in treated males and controls.	
2	1.01	1.03		
6	0.97	0.79		
18	0.87	1.47		
54	0.38**	0.48		

Females

Dose(ppm)	Neutrophils X $10^3$ /cmm		MCHC%		Hb (g/dl)	
	wk 6	wk 13	wk 6	wk 13	wk 6	wk 13
0	0.30	0.30	31.3	28.6	15.4	13.7
2	0.28	0.27	31.0	29.8**	15.1	14.9*
6	0.31	0.19	30.1*	29.3*	14.4	14.6*
18	0.26	0.23	30.0*	29.6*	14.8	14.6*
54	0.36	0.46	31.1*	30.5	15.5	14.7

\* Significant at  $p < 0.05$  as compared to controls.

\*\* Significant at  $p < 0.01$  as compared to controls.

Analysis of the difference between controls and 54 ppm dose group was not performed due to high mortality in that group, according to the test report.

Males showed a decrease in neutrophil numbers at 54 ppm at both 6 and 13 weeks. At 6 weeks the drop was dose-related whereas at 13 weeks the drop was apparently not dose-related. Females showed a slight increase in neutrophils at 54 ppm at both 6 and 13 weeks but the increase was minimal.

Males and females showed minimal polychromasia at week 13. This effect appeared in male controls but not in female controls. This finding could suggest an increase in numbers of reticulocytes or an increase in staining of macrocytes which may be compound related.

### Biochemistry

Several biochemistry values in dosed animals were significantly different from control values. Glucose levels in females at weeks 6 and 13 were significantly lowered at 6, 18, and 54 ppm., in the opinion of our statistician (Litt). Glutamic pyruvic transaminase (GPT) in females at 13 weeks was significantly elevated at 54 ppm. The value showed great variability (very large standard deviation), and may be due to generalized toxicity at the high dose level. In males, lowered alkaline phosphatase (AP) was seen at 6 and at 54 ppm in week 13 and lowered GPT at 2 and at 54 ppm. These values are indicated by the symbol "°". The effect on biochemistry values appears to be compound-related but not necessarily dose-related.

Table VII. Group Mean Biochemistry Values

#### Females

Dose (ppm)	Glucose (mg/dl)		Protein (g/dl)		AP (mU/ml)		GPT (mU/ml)	
	wk 6	wk 13	wk 6	wk 13	wk 6	wk 13	wk 6	wk 13
0	172	164	5.5	5.6	127	90	23	30
2	163	168	5.4	5.6	146	118	24	26
6	151**	155	5.5	5.5	145	85	23	32
18	151**	157	5.4	5.7	157	109	27	30
54	152**	140	5.3*	5.4	130	98	28	46

#### Males

0	172	192	5.5	5.4	104	86	35	57
2	163	187	5.6	5.4	107	79	33	27°
6	175	190	5.5	5.6	91	63°	32	53
18	186	190	5.6	5.7	128	90	33	40
54	165	177	5.5	5.5	94	65°	30	21°

\* Significant at  $p < 0.05$  as compared to control value.

\*\* Significant at  $p < 0.01$  as compared to control value.

### Macroscopic Pathology

Macroscopic pathology examination in males revealed 5/20 (25%) at 54 ppm with congested lungs, 2/20 (10%) with pale areas in the liver, 2/20 (10%) with pale kidneys, and 2/20 (10%) with pale spleen, whereas the incidence was zero in all other groups. The examination in females showed 2/20 (10%) with congested lungs at 54 ppm.

### Histopathology

Lungs Females at 54 ppm showed slight vascular congestion (6/20 at 54 ppm vs. 0/20 in controls) and recent hemorrhage (3/20 at 54 ppm vs. 0/20 in controls) possibly due to terminal sacrifice. All dose groups including controls showed slight to minimal extramedullary hemopoiesis and several showed autolysis of the gastrointestinal tract, probably due to post mortem release of enzymes.

### Discussion

The NOEL for lethality is 18 ppm. There was no indication of the mechanism of lethality in the test report. The compound is a known central convulsant, however, this effect accounted for only two of the deaths noted on test.

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