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

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MAY 16 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Subchronic Oral Toxicity (90-day) Study on Endosulfan in the Rat

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

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

THROUGH: Marcia Van Gemert, Ph.D., Head
Review Section III

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

Compound: Endosulfan Technical Tox. Chem.: 420

Registration #: 154111 Registrant: American Hoechst

Accession No.: 257727 Tox. Project #: 490

Study Identification: Endosulfan-Active Ingredient Technical 13-week Toxicity Study in Rats Followed by a 4-week Withdrawal Period (Final Report), Barnard, A.V., et.al., Unpublished study conducted by Huntingdon Research Centre plc, Huntingdon, Cambridgeshire, England for Hoechst Aktiengesellschaft, Pharma Forschung Toxicologie, Frankfurt, West Germany, March 25, 1985.

Action Requested: Review of the 13 week toxicity study in rats with Endosulfan Technical, which was identified as a "data gap" in the 1982 Endosulfan Registration Standard.

Conclusions from Data Evaluation Report:

Five groups of 25 male and 25 female CD Sprague-Dawley rats received 0, 10, 30, 60, and 360 ppm Endosulfan Technical in the diet for 13 weeks. Twenty of each group were sacrificed at 13 weeks and 5 were sacrificed after an additional 4-week recovery period. There was no significant mortality during the test period. Alopecia was the only sign of toxicity. The NOEL for this effect was 30 ppm.

Bodyweight was marginally lowered by the test compound in males and females at 360 ppm. Food consumption and food efficiency were not significantly affected by the test compound in females. Males showed slightly inferior food efficiency through the last two thirds of the test period, as reflected by lowered body weight for this period. The most notable change observed among the hematology and clinical chemistry parameters tested was lowered red blood cell count, which becomes important when coupled with kidney histopathology, as discussed below. Discolored urine at 60 and 360 ppm may have been the result of the hematopoietic effects. Although the exact nature of the discoloration was not discovered, it should be considered along with the pigmentation observed in the proximal convoluted tubules in significant amounts at 360 ppm and in lesser amounts at all other doses. Notable increases in absolute and relative organ weights were found at 13 and 17 weeks at 60 and 360 ppm in the spleen and kidney of males.

Discussion of Kidney Effects: The observation of unusual pigmentation in the proximal convoluted tubules of the kidney (yellowish discoloration and granular/clumped pigment), the discolored urine and the depressed hematology values indicate there may be an effect of Endosulfan on the hematopoietic system. These effects were observed in the reproduction studies (EPA Accession Nos. 256126, 256127, 257727) in the rat in which the histopathology examination revealed pigmentation and lysosomal inclusions in kidney proximal convoluted tubules but in which hematology values were not examined. The Addendum to the Reproduction study examined the histopathology of the kidneys in order to identify the source of the pigmentation, an attempt which failed. The chemical nature of the granules and yellowish discoloration were not determined. The subchronic (13-week) study in mice (EPA Accession No. 256114) found lowered hematology values at 6 weeks in females and elevated values at 13 weeks. The evidence from the present study indicates that Endosulfan apparently causes some destruction of red blood cells with subsequent discoloration of the kidney cells and urine during the process of elimination of the by-products of the breakdown-a mild hemoglobinuria. The spleen of females at 360 ppm showed a minimal/moderate hemosiderosis indicating increased iron in the blood, and supporting the above evidence showing probable destruction of red blood cells.

A No Observable Effects Level was not found in this study: Kidney effects observed in males at all doses: granular/clumped pigment of proximal convoluted tubules; and hematology effects observed at all doses: lowered hemoglobin in females at week 13 and elevated platelet count in males at week 13.

This effect should be carefully monitored in future studies with Endosulfan, in the long term studies to determine the exact cause of the discoloration of proximal convoluted tubule cells, whether the effect is permanent or reversible over the long term, whether there is an effect on long term survival and health of the animals, and particularly to find the level where no effects are seen.

Classification: Core Minimum (Acceptable)

Reviewed by: Margaret L. Jones, M.S.
Section III, Tox. Branch, (TS-769)

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Secondary reviewers: Marcia Van Gemert, Ph.D., Section Head;
Clint Skinner, Ph.D.; Alan Katz, D.A.B.T.
Section III, Tox. Branch, (TS-769)

DATA EVALUATION REPORT

Study Type: Subchronic (90-day) Oral Toxicity in the Rat

Accession Number: 257727

Test Material: Endosulfan - Technical

Synonyms: Thiodan®, Thionex®

Study Number: A 30700, HRC Report Number: HST 230/84176

Registrant: American Hoechst

Sponsor: Hoechst Aktiengesellschaft, Pharma Forschung Toxikologie
6230 Frankfurt am Main 80, Federal Republic of Germany

Testing Facility: Huntingdon Research Centre plc, Huntingdon,
Cambridgeshire, England

Title of Report: Endosulfan - Active Ingredient Technical (Code: HOE oo2671 0I ZD97 0003) 13-Week Toxicity Study in Rats Followed by a 4-Week Withdrawal Period (Final Report)

Authors: Barnard, A.V., et.al.

Report Issued: March 25, 1985

Conclusions: Five groups of 25 male and 25 female CD Sprague-Dawley rats received 0, 10, 30, 60, and 360 ppm Endosulfan in the diet for 13 weeks. Five animals from each group were held for an additional 4-week withdrawal period and were sacrificed at 17 weeks. There was no significant mortality during the test period. Alopecia was the only sign of toxicity. The NOEL for this effect was 30 ppm.

Bodyweight was marginally lowered by the test compound in males and females at 360 ppm. Food consumption and food efficiency were not significantly affected by the test compound in females. Males showed slightly inferior food efficiency through the last two thirds of the test period, as reflected by lowered body weight for this period. The most notable change observed among the hematology and clinical chemistry parameters tested was lowered red blood cell count, which becomes significant when coupled with kidney histopathology, as discussed below. Discolored urine at 60 and 360 ppm may have been the result of the hematopoietic effects. Although the exact nature of the discoloration was not discovered, it should be considered along with the pigmentation observed in the proximal convoluted tubules in significant amounts at 360 ppm and in lesser amounts at all other doses. Notable increases in absolute and relative organ weights were found in males at 13 and 17 weeks at 60 and 360 ppm in the spleen and kidney.

Discussion of Kidney Effects: The observation of unusual pigmentation in the proximal convoluted tubules of the kidney (yellowish discoloration and granular/clumped pigment), the discolored urine and the depressed hematology values indicate there may be an effect of Endosulfan on the hematopoietic system. These effects were observed in the reproduction studies (EPA Accession Nos. 256126, 256127, 257727) in the rat in which the histopathology examination revealed pigmentation and lysosomal inclusions in kidney proximal convoluted tubules but in which hematology values were not examined. The Addendum to the Reproduction study examined the histopathology of the kidneys in order to identify the source of the pigmentation, an attempt which failed. The chemical nature of the granules and yellowish discoloration were not determined. The subchronic (13-week) study in mice (EPA Accession No. 256114) found lowered hemoglobin values at 6 weeks in females and elevated values at 13 weeks. The evidence from the present study indicates that Endosulfan apparently causes some destruction of red blood cells with subsequent discoloration of the kidney cells and urine during the process of elimination of the by-products of the break-down- a mild hemoglobinuria. The spleen of females at 360 ppm showed a minimal/moderate hemosiderosis indicating increased iron in the blood, and supporting the above evidence showing probable destruction of red blood cells.

A No Observable Effects Level was not found in this study: Kidney effects observed in males at all doses: granular/clumped pigment of proximal convoluted tubules; and hematology effects observed at all doses: lowered hemoglobin in females at week 13 and elevated platelet count in males at week 13.

These effects should be carefully monitored in future studies with Endosulfan, in the long term studies to determine the exact cause of the discoloration of proximal convoluted tubule cells, whether the effect is permanent or reversible over the long term, whether there is an effect on long term survival and health of the animals, and particularly to find the level where no effects are seen.

Classification: Core Minimum (Acceptable)

A. Materials

Test Compound: Endosulfan-Technical; 6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide; "...buff-colored flakes."(p 2, Test Report A 30700); Batch No: HOE 002671 OI ZD97 0003; 97.2% pure; no contaminants listed.

Test Animals: Male and female caesarian derived Sprague-Dawley rats from the Charles River Laboratory, Portage, Mich., USA were 28 days old on the day of delivery. The weight of males ranged from 164-221g (group mean weights ranged from 191-195g) and weight of females ranged from 126-174g (group mean weights ranged from 148-153g) at initiation, 13 days after delivery. The rats were 41 days old when placed on test diets (7/27/86).

B. Study Design

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Animal Assignment: 250 male and female rats were randomly assigned to 5 groups of 25 which received 0, 10, 30, 60, or 360 ppm in the test diet. Five animals were housed in each cage. Preselection of animals was made by numbering animals consecutively and sacrificing the survivors among the first 20 of each group (eg: 1-20) at 13 weeks and the last 5 (eg: 21-25) at 17 weeks, after a 4-week withdrawal period.

Diet Preparation: The test compound was administered by mixture with the diet. The high dose was prepared by first dissolving the test compound in acetone and dispersing it with corn oil in the feed. Other doses were prepared by dilution with additional feed. (Each dose level, therefore, contained different residues of acetone and vehicle.) Controls received laboratory diet mixed with acetone and corn oil. Dietary mixtures were analysed to determine homogeneity and stability. Samples were taken in weeks 1,6, and 13 of the test period. Analyses were checked by the HRC Quality Assurance Unit. Results are shown in the following tables. Table 2 shows the mean achieved intakes of test substance in mg/kg bodyweight/day.

Results of Stability Analysis

Dose(ppm)		Storage Time (days) 9	18
10	(1)	9.53	9.90
	(2)	9.64	10.3
360	(1)	347	341
	(2)	346	344

Results of Homogeneity Analysis

Dose(ppm)	Range of 6 Samples(ppm)	Mean
10	9.82-10.3 (S.D.=0.170)	10.0
360	349-373 (S.D.=7.81)	363

From these results, there were no apparent problems with homogeneity or stability.

Animals received Spratt's Laboratory Diet No. 2 and tap water ad libitum.

Statistical Analysis: The following methods were used to analyse the results statistically: Analysis of variance (for significance of intergroup differences in food consumption, bodyweight, and water consumption using group mean figures for food consumption analysis, and individual data for other analyses), Student's "t" test (for intergroup comparisons), Bartlett's test, Kruskal-Wallis, or analysis of ranks (for clinical pathology in which relative frequency of the mode was less than 75%), Analysis of variance and Student's "t" test and William's test (for dose-related responses), Mantel's and Fisher's tests (for values where relative frequency of the mode was greater than 75%), Analysis of variance or analysis of covariance (for organ weights- with body weight as covariate), Williams' test (for intergroup comparison where dose-related response was found.

C. Methods and Results

Physical Examinations: Animals were inspected twice daily for mortality and once daily for clinical signs of toxicity during the first four weeks of the study. Thereafter, clinical observations were made once weekly. Indirect ophthalmoscopic examination took place at pretest and at 13 weeks. Neurological examinations were made at pretest on 10 males and 10 females from each dose group and at weeks 2, 6, and 13 of the study on 10 males and 10 females from the control and high dose group. At 13 weeks the animals were also checked for grip reflex and ataxia.

Results: The only apparent sign of toxicity was in females which received 60 and 360 ppm Endosulfan. These females showed increased hair loss in the dorsal/scapular/cervical regions, as compared to controls. The hair loss regressed by the end of the 4-week recovery period. Males did not show hair loss. Table 1 shows the incidence of alopecia in females.

Three deaths occurred in females during weeks 1-13. One control female died during week 5 and one female receiving 60 ppm died during week 6. These deaths were attributed to anesthetic shock during blood sampling (see Hematology section for a description of the sampling method). The third female who received 360 ppm died in week 10. That female showed minimal congestion of the thymus, minimal lymphoid aggregates in the lung, yellowish pigment in the tubules of the cortex of the kidneys, a small area of mononuclear cells in the liver, and some swollen nerve fibers in the spinal cord. The cause of death was not identified. No unscheduled deaths occurred in any of the male rats.

Table 1
Incidence of Hair Loss in Female Rats

Dose (ppm)	0		10		30		60		360	
	week		week		week		week		week	
	13	17	13	17	13	17	13	17	13	17
# examined	19	5	20	5	20	5	19	5	19	5
# w. hair loss	1	0	4	1	1	1	9	3	7	1

No ocular changes were found in any control or treated animals. Neurological examination revealed no effects which were related to treatment.

No significant clinical signs or mortality were attributed to the test compound.

Body weight: Test animals were weighed prior to distribution to groups, one week later at the start of treatment, and weekly for the remainder of the test period.

Results: Group mean body weights of males and females at 360 ppm were slightly lower than control weights from weeks 6 through 17. Bodyweight at 360 ppm was marginally lower (<0.05%) than controls from week 6-13 for males and from week 1-13 for females. Males at 360 ppm gained 3-6 times less weight over the 13 week test period than did other dose groups compared to controls. Females at 360 ppm gained significantly ($p<0.001$) less weight than did other dose groups compared to controls in weeks 0-2 only. From week 14-17 (throughout the recovery period) group mean body weights for males formerly receiving 360 ppm were 12-13% lower than controls. (Since only 5 animals per group were held through the withdrawal period, statistical analysis of this difference would not be meaningful.)

Conclusion: Bodyweight was marginally lowered by the test compound in males and females at 360 ppm.

Food consumption and efficiency: Food consumption was measured weekly for each cage of 5 rats. Intake per rat was calculated as the difference between the amount of food given to and left in each cage divided by the number of live animals per cage. Efficiency was calculated "where appropriate" (Test Report HST/ 230, p. 6) as food consumed per unit gain in body weight.

Results: Females receiving 360 ppm Endosulfan showed a mean food consumption significantly ($p<0.001$) lower than controls during the first two weeks of administration of the test substance. Except for this difference, group mean food intake over the 13-week test period and through the 4-week recovery period in each dose group was similar to controls.

Efficiency of food utilization was inferior in females receiving 360 ppm during week 1. Males receiving 360 ppm also showed inferior food efficiency values between weeks 4 and 13.

Conclusion: Food consumption and food efficiency were not significantly affected by the test compound in females. Males showed slightly inferior food efficiency through the last two thirds of the test period, as reflected by lowered body weight for this period.

Hematology and Clinical Chemistry: Blood was collected before treatment on a health check group consisting of 10 males and 10 females. At 6 and 13 weeks blood was collected for hematology investigation under light ether anaesthesia from the orbital sinus of 10 males and 10 females from each dose group. In a similar manner, at weeks 6 and 12, blood was collected for biochemistry investigation. Samples were also taken at the end of an additional 4-week recovery period from 5 males and 5 females.

The following hematology parameters were measured:

Hematocrit (HCT)g	Total plasma protein g
Hemoglobin (Hb)g	Leukocyte differential count
Leukocyte count (WBC)g	Mean corpuscular Hb conc. (MCHC)
Erythrocyte count (RBC) g	Mean corpuscular volume (MCV)
Platelet countg	Packed cell volume (PCV)
	Reticulocyte count
	Thrombotestg (ref: Owren, P.A., Lancet, 1959, ii, 754)

The following clinical chemistry parameters were measured:

Electrolytes:	Other:
Calciumg	Albumin g
Chlorideg	Blood creatinineg
Magnesiumg	Blood urea nitrogeng
Inorganic Phosphorousg	Cholesterolg
Potassiumg	Globulins
Sodiumg	Glucoseg
Enzymes:	Total Bilirubing
Alkaline phosphatase	Total Proteing
Cholinesterase (plasma&RBC)	Triglycerides
Total Lactic acid dehydrogenase	
Serum alanine aminotransferase (also SGPT)g	
Serum aspartate aminotransferase (also SGOT)g	
gamma-glutamyl transpeptidase	

g- indicates parameter suggested in the Pesticide Assessment Guidelines for Hazard Evaluation, 1982 (Subpart F).

Results: Hematology- Values that showed significant changes as compared to controls are presented in Table 2. Of note were hemoglobin and RBC counts. At 360 ppm the values were significantly ($p<0.01$ and $p<0.05$-see Table 2) lower than controls in males and females at both weeks 6 and 13. Hemoglobin and RBC values in males remained significantly lower than controls after withdrawal (week 17), whereas females returned to normal. At 6 weeks, lowering of hemoglobin and RBC counts at 60 ppm was less pronounced than at 360 ppm but still significantly lower ($p<0.01$ and $p<0.05$-see Table 2) at week 6. At week 13, females showed significantly ($p<0.05$) lower hemoglobin and males showed significantly($p<0.05$) lower RBC counts.

The lowered RBC values are meaningful when compared with the kidney histopathology results. The discoloration and granular pigment observed in the proximal convoluted tubules of the kidneys at all doses in varying degrees (see Tables 7 and 8) are the result of possible hematopoietic effects of Endosulfan. For further description of kidney effects, refer to the section on microscopic pathology.

Clinical Chemistry - Significant changes in biochemistry values are shown in Table 3. Most of the changes appear at 360 ppm. Although statistically significant, most of the changes do not demonstrate important toxicity of Endosulfan.

Cholinesterase values are given in Table 3. Significant changes from control values were limited to females at 360 ppm, involving the following parameters:

blood cholinesterase lowered at week 12
plasma cholinesterase lowered at week 6 and 12
brain cholinesterase lowered at week 13

All values returned to normal by the end of week 16 (during withdrawal).

As with the majority of hematology values, no significant toxicity was demonstrated in the biochemistry values from this study.

Table 3

Hematology- group mean values for males and females[1]

Group (ppm)	week 6		week 13		week 17 (after withdrawal)	
	PCV %					
	m	f	m	f	m	f
0	51	48	52	48	52	49
10	52	49	53	48	52	49
30	51	49	52	48	52	49
60	51	48	51	50	53	44
360	49*	48	49**	48	49**	47
	Hb g/dl					
	m	f	m	f	m	f
0	14.6	13.7	16.1	15.0	16.6	14.8
10	14.9	13.9	16.5	14.5*	16.0	15.2
30	14.1*	13.4	16.1	14.4*	16.0	15.9
60	13.8**	12.8**	15.9	14.5*	15.7*	13.7
360	13.4**	12.4**	15.0**	14.3**	15.0**	14.5
	RBC x 10^6/cmm					
	m	f	m	f	m	f
0	7.9	6.8	9.4	7.9	8.8	7.1
10	7.9	6.9	9.4	7.5	8.4	7.4
30	7.5*	6.7	9.2	7.7	8.4	7.6
60	7.2*	6.4*	8.8*	7.7	8.2	6.6
360	7.0*	6.1**	8.6**	7.3**	7.8**	7.0

1. From Table 6, Test Report HST/230, pp. 35-40.
* $p<0.05$ as compared to controls
** $p<0.01$ as compared to controls

Hematology - group mean values for males and females (continued)[1]

	week 6		week 13		week 17 (after withdrawal)	
	MCHC %					
	m	f	m	f	m	f
0	28.8	28.6	30.9	31.0	31.9	30.3
10	28.9	28.6	31.4	30.4	30.6*	30.9
30	27.7**	27.3*	30.7	29.8**	30.2**	32.4*
60	27.3**	26.9**	31.0	29.2**	30.3**	30.8*
360	27.3**	26.2**	30.6	29.8**	30.3**	30.9

Group (ppm)	MCV fl					
	m	f	m	f	m	f
0	65	71	56	61	59	69
10	66	70	56	64	63	66*
30	68**	73	57	63	63	64*
60	70**	74**	58	65*	63	68*
360	70**	78**	57	66*	64*	67*

	Plts x10^3/cmm				TT secs			
	week 6		week 13		week 6		week 13	
	m	f	m	f	m	f	m	f
0	700	652	413	589	25.9	23.3	24.7	22.3
10	708	591*	595**	563	26.3	23.2	27.6*	21.3
30	703	553*	588**	587	26.0	22.4	26.7*	22.7
60	661	538*	540**	557	27.1	22.3	28.0*	20.9
360	667	514*	573**	554	25.9	22.8	24.9*	22.0

1. From Table 6, Test Report HST/230, pp. 35-40.

* $p<0.05$ as compared to control values

** $p<0.01$ as compared to control values

Table 4

Biochemistry- group mean values[1]

Group (ppm)	Total protein (g/dl)				Albumin (g/dl)				Globulin (g/dl)			
	week 6		week 12		week 6		week 12		week 6		week 12	
	m	f	m	f	m	f	m	f	m	f	m	f
0	6.6	7.0	6.7	7.3	3.5	3.9	3.2	3.7	3.1	3.2	3.5	3.6
10	6.7	7.0	6.8	7.4	3.5	3.6	3.1	3.6	3.2	3.3	3.7	3.8
30	6.8	6.8	7.0	7.4	3.4	3.9	3.2	3.7	3.4*	2.9	3.8*	3.7
60	6.8	6.8	7.1	7.4	3.4*	3.7	3.3	3.7	3.4*	3.0	3.8*	3.7
360	6.9*	7.0	6.8	7.1	3.4*	3.7	3.1*	3.7	3.5**	3.3	3.8*	3.7

Group (ppm)	GPT (mU/ml)				GOT (mU/ml)			
	week 6		week 12		week 6		week 12	
	m	f	m	f	m	f	m	f
0	26	16	28	15	56	55	63	45
10	28	20	32	18	58	60	67	48
30	26	17	29	21*	58	51	63	47
60	22	18	26	21*	60	56	62	51
360	20**	22*	22*	19*	54	63	49**	51

Group (ppm)	K (mEq/l)				P (mEq/l)			
	week 6		week 12		week 6		week 12	
	m	f	m	f	m	f	m	f
0	3.7	4.0	3.4	3.3	3.8	3.5	3.5	2.9
10	3.7	3.7	3.7	3.4	3.7	3.2	3.7	2.9
30	3.9	3.8	3.6	3.4	3.9	3.3	3.8**	2.9
60	3.8	3.6**	3.8*	3.6*	4.0	3.3	3.8**	2.9
360	3.9	3.5**	3.8*	3.6*	3.8	3.3	3.9**	3.2*

Group (ppm)	Chol. (mg/dl)				Lipid (mg/dl)			
	week 6		week 12		week 6		week 12	
	m	f	m	f	m	f	m	f
0	53	68	58	66	341	370	279	290
10	53	72	57	62	381	338	277	277
30	63	77	57	68	419	381	288	307
60	62	75	61	66	351	322	294	99
360	61	93**	66	84**	397	426*	324	53**

1. From Table 7, Test Report ST 230, pp.41-45.
* $p<0.05$ in comparison with control value
** $p<0.01$ in comparison with control value

Biochemistry- group mean values (continued)

Cholinesterase values

Blood Cholinesterase
(umol/g/min)

Group	males week		females week		females week
(ppm)	5	12	5	12	16
0	1.68	1.75	1.65	1.89	2.41
10	1.63	1.87	1.66	1.65	2.21
30	1.51	1.71	1.57	1.79	2.07
60	1.63	1.87	1.57	1.77	2.23
360	1.57	1.78	1.64	1.66*	2.28

* $p<0.05$ in comparison with control value

Plasma Cholinesterase
(umol/g/min)

Group	males week		females week		females week
(ppm)	5	12	5	12	16
0	0.42	0.47	1.21	1.61	1.42
10	0.43	0.46	1.20	1.55	1.63
30	0.44	0.50	1.11	1.57	1.39
60	0.42	0.45	1.13	1.70	1.42
360	0.42	0.47	0.79**	0.95**	1.44

** $p<0.01$ in comparison with control value

Brain Cholinesterase
(umol/g/min)

Group	males week	females week	
(ppm)	13	13	16
0	7.06	5.31	4.92
10	7.66	5.51	4.84
30	5.88	6.08	5.04
60	6.32	6.30	4.54
360	7.00	6.35*	4.74

* $p<0.05$ in comparison with control value

Urinalysis - Urine was collected overnight from 10 males and 10 females at weeks 4 and 13. Samples were again collected at the end of the withdrawal period from 5 males in each group to estimate proteins, ketones, and color.

The following urine values were measured:

Appearance[g] (color)	Glucose[g]
Volume[g]	Ketones[g]
Specific gravity[g]	Bilirubin[g]
pH	Blood[g]
Sediment (microscopic)[g]	Hemoglobin
Protein[g]	Urobilinogen
	Reducing substrate

g- indicates parameter suggested in the Pesticide Assessment Guidelines for Hazard Evaluation, 1982 (Subpart F).

Results: Proteins were present in the urine of males at all doses, including controls at weeks 4, 13, and after the withdrawal period, at week 17. Group mean protein levels were significantly increased over controls in males at 360 ppm at week 13, as shown in Table 4.

Urine of males was darker than controls at 60 and at 360 ppm at week 13-"medium straw" at 60 ppm and "dark straw" at 360 ppm v. "light straw" for controls. After withdrawal, no difference in color between urine of treated and control groups was seen. Urine of females was darker than controls at 360 ppm-"medium straw".

The sedimentation test to find occult blood and other substances in the urine showed polymorphonuclear leukocytes in males at week 6 and "few organisms" in males and females at weeks 6 and 13, but apparently no RBC.

There was a significant increase over controls in urine volume along with significantly lowered specific gravity in males at 360 ppm when tested at week 4.

Ketones were positive in the urine of males at all doses, including controls at both weeks 4 and 13. At week 13, males at 60 and 360 ppm showed higher qualitative levels of ketones than controls.

Females showed no differences from controls in any of the urine parameters tested at week 4 or 13 and were therefore not tested at week 17.

There were apparently no significant effects produced by Endosulfan which appeared in the urine.

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Table 5.

Urinalysis- group mean values[1]

Protein (mg/dl)

Group (ppm)	week 4		week 13		week 17[2]	
	m	f	m	f	m	f
0	84	0	28	0	28	(
10	70	0	35	0	74	(not
30	72	0	39	0	24	(
60	63	0	43	0	48	(tested
360	96	0	53*	0	42	(

1. From Table 9, Test Report HST 230, pp. 51-55.
2. Only 5 animals per group; statistical analysis not performed
* $p<0.05$ as compared to controls

Sacrifice and Pathology- The three females that died (one control in week 5, one at 60 ppm in week 6, and one at 360 ppm in week 10) and those that were sacrificed at week 13 (19 females from groups receiving 0, 60, and 360 ppm, and 20 animals from all the remaining groups) and at week 17 (5 animals from each dose group) were subject to gross pathological examination and the following tissues were collected for histological examination. Those organs with * were weighed.

Digestive system
- Tongue
- Salivary glandsg
- Esophagusg
- Stomachg
- Duodenumg
- Jejunumg
- Ileumg
- Cecumg
- Colong
- Rectumg
- Liverg*
- Pancreas

Respiratory
- Tracheag
- Lung
- Larynx
- Pharynx

Cardiovasc./Hematol.
- Aortag
- Heartg*
- Lymph noedsg
- Spleeng
- Thymusg

Urogenital
- Kidneysg
- Urinary bladderg
- Testesg*
- Epididymides*a
- Prostate
- Seminal vesicles
- Ovaries*
- Uterusg*
- Vagina

Neurologic
- Braing*
- PItuitaryg*
- Spinal cord (3 levels)g
- Eyes (optic n.)g

Glandular
- Adrenalsg*
- Mammary glandg
- Thyroidsg*

Other
- Boneg
- Skin
- Skeletal muscleg
- Brain cholinesterase
- Harderian Gland
- Head (nasal cavity, sinuses, oral cavity, nasopharynx, middle ear teeth, lachrymal gland, zymbals gland)

a. This organ was weighed only, not examined histologically.
g- indicates parameter suggested in the Pesticide Assessment Guidelines for Hazard Evaluation, 1982 (Subpar. F).

15

Results-

a. Macroscopic examination-

13 weeks- Enlarged kidneys were noted in 11/20 males at 360 ppm and in 2/20 males at 60 ppm. Enlargement of the liver was found in 3/20 males at 360 ppm. No kidney or liver enlargement were found in controls. Alopecia was noted in females at all doses but in significantly increased amounts at 60 (9/19) and at 360 ppm (7/19). (See Table 1)

17 weeks- Enlarged kidneys were found in 1/5 males at 360 ppm. Enlarged cervical lymph nodes were found in females in the following numbers: 0 ppm-2/5, 10 ppm-3/5, 30 ppm-0/5, 60 ppm-5/5, 360 ppm-5/5. Alopecia was observed in 3/5 females at 60 ppm and in 1/5 females at 360 ppm. Three of five male controls and 1/5 female controls exhibited alopecia.

b. Absolute and Relative Organ weights- Tables 6 and 7 show the absolute and relative weights of selected organs.

13 weeks- Significantly increased mean liver and kidney weights were found in all animals at 360 ppm. Mean kidney weight in males at 60 ppm was also significantly increased. Epididymides were significantly increased in weight at 360 ppm. Brain weights in females were significantly increased at 60 and at 360 ppm. The levels of significance are indicated in Table 5. Relative organ weights compared to controls were affected at 360 ppm, as follows. In males, relative liver weight was increased 21%, relative kidney weight was increased 37.5%, and relative weight of epididymides was increased 29%. In females, relative liver weight was increased 32%, relative kidney weight was increased 14%, and relative weight of ovaries was decreased 9% at 360 ppm.

17 weeks- Brain weights in males at 60 and 360 ppm were increased, as were spleen weights at 30, 60, and 360 ppm, and kidney weights were significantly increased at 360 ppm. In females, adrenal weight was increased at 360 ppm. Relative organ weights compared to controls were affected at 360 ppm, as follows. In males, relative spleen weight was increased 50%, relative brain weight was increased 21% liver weight was increased 19%, kidney weight was increased 36%, and epididymides were increased by 29%. No important changes in relative organ weights were observed in females.

Notable increases in absolute and relative organ weights were found in males at 13 and 17 weeks at 60 and 360 ppm in the spleen and kidney.

c. Microscopic examination-

13 weeks- At terminal (13 week) microscopic examination, effects were noted in the kidneys, lungs, and spleen. Kidney effects were the most remarkable finding. The numbers of rats showing the various pigment abnormalities are shown in Table 8, adopted from Tables A, B, C, and D (pp. 23-25) of Test Report HST/230. Yellowish discoloration in the proximal convoluted tubule cells was found in all male rats at the 13 week sacrifice. The discoloration varied from trace to moderate and was dose related in severity and incidence. Female rats showed a dose-related increase in numbers showing trace discoloration. Cortical areas of tubular basophilia were noted in males at all dose levels at terminal sacrifice. The incidence is shown in Table 9, adopted from Table 16 of Test Report HST/230, pp 68-75.

Vacuolated nerve fibers in the spinal cord were found at 360 ppm in males at terminal sacrifice.

In the lung, medial calcification of the blood vessels was observed in all animals, including controls. The effect was apparently not dose related and is not considered related to the compound, since the frequency in males decreased at the high dose. (This effect is noted because it is relatively rare.)

In the spleen, minimal to moderate hemosiderosis was found in 11/19 females at 360 ppm and in 7/19 female controls. This was accompanied by significant lowering of RBC in females at 360 ppm. No significant hemosiderosis was found in male spleens at 360 ppm, although RBC were lowered at this dose.

17 weeks- The kidney effects which persisted through the withdrawal period are shown in the bottom section of Table 5. Yellowish discoloration persisted in males at 360 ppm in trace amounts, and was also observed at 10 and at 30 ppm. The discoloration disappeared in females after withdrawal. Granular/clumped pigment persisted in males and females in trace amounts- in males at 30 and 60 ppm and in females at 60 and 360 ppm. No hemosiderosis was observed in the spleen after the recovery period in males or females.

The most important microscopic observation was in the kidney where pigmentation of the proximal convoluted tubule cells was noted. The effect persisted to withdrawal in males at all doses and in females at 60 and 360 ppm. No NOEL for this effect was found. As noted in the conclusion section, this effect was previously reported in the reproduction study.

Table 6

Mean Absolute Weights of Selected Organs (g)[1]

005115

Week 13

males	Group (ppm)	Bodyweight	Liver	Kidneys	Spleen	Epididymides	Testes
	0	495	20.2	4.1	0.70	1.21	3.30
	10	503	21.7	4.1	0.74	1.27	3.38
	30	505	21.6	4.3	0.75	1.30	3.42
	60	500	21.5	4.5**	0.71	1.26	3.39
	360	481	24.6**	5.1**	0.75	1.32*	3.55

females	Group (ppm)	Bodyweight	Liver	Kidneys	Ovaries	Brain
	0	292	12.4	2.6	100	1.84
	10	288	11.6	2.5	95	1.84
	30	293	11.5	2.5	98	1.86
	60	288	11.5	2.5	89	1.93**
	360	286	15.3**	2.8**	88	1.96**

Week 17

males	Group (ppm)	Bodyweight	Liver	Kidneys	Spleen	Epididymides	Testes
	0	532	23.6	4.2	0.63	1.334	3.34
	10	541	23.7	4.2	0.73	1.184	3.24
	30	499	21.6	4.0	0.81*	1.227	3.58
	60	513	20.3	4.1**	0.76*	1.347	3.58
	360	468	20.6	4.5**	0.82*	1.298	3.48

females	Group (ppm)	Bodyweight	Liver	Kidneys	Ovaries	Brain
	0	293	12.2	2.4	86	1.92
	10	292	12.9	2.6	92	1.88
	30	317	12.5	2.7	87	1.88
	60	318	12.4	2.7	65	1.88
	360	305	13.8	2.6	90	1.94

1. From Tables 11 and 13, Test Report HST 230, pp. 58-59 and 62-63.

* $p<0.05$ as compared to controls

** $p<0.01$ as compared to controls

Table 7

Relative Weights of Selected Organs (from Mean Organ Weights)[1]

Week 13

males	Group (ppm)	Bodyweight (g)	Liver	Kidneys	Spleen	Epididymides	Testes
	0	495	0.042	0.008	0.0014	0.0021	0.0068
	10	503	0.044	0.008	0.0015	0.0026	0.0069
	30	505	0.041	0.008	0.0015	0.0025	0.0067
	60	500	0.045	0.009	0.0014	0.0026	0.0070
	360	481	0.051	0.011	0.0016	0.0027	0.0073

females	Group (ppm)	Bodyweight (g)	Liver	Kidneys	Ovaries
	0	292	0.041	0.0088	0.339
	10	288	0.041	0.0090	0.335
	30	293	0.040	0.0088	0.344
	60	284	0.044	0.0095	0.334
	360	286	0.054	0.0100	0.309

Week 17

males	Group (ppm)	Bodyweight (g)	Liver	Kidneys	Spleen	Epididymides	Testes
	0	532	0.042	0.0076	0.0012	0.0024	0.0063
	10	541	0.041	0.0074	0.0014	0.0021	0.0060
	30	499	0.045	0.0083	0.0016	0.0025	0.0072
	60	513	0.041	0.0083	0.0015	0.0027	0.0070
	360	468	0.050	0.0103	0.0018	0.0031	0.0074

females	Group (ppm)	Bodyweight (g)	Liver	Kidneys	Ovaries
	0	293	0.044	0.0086	0.298
	10	292	0.047	0.0096	0.319
	30	317	0.039	0.0082	0.278
	60	318	0.038	0.0083	0.207
	360	305	0.046	0.0087	0.299

1. From Tables 11 and 13, Test Report HST 230, pp. 58-59 and 63-63.

Table 8

Incidence of Abnormalities in Kidney Pigmentation at 13 week Examination[1]

	males					females				
Dosage	0	10	30	60	360	0	10	30	60	360
Yellowish discolored cells in proximal convoluted tubule										
traces	0	14	17	7	0	0	0	1	8	19
minimal	0	0	2	9	7	0	0	0	0	0
moderate	0	0	0	3	13	0	0	0	0	0
Granular/clumped pigment in straight and/or proximal convoluted tubule										
traces	0	0	0	3	0	1	0	0	0	17
minimal	0	0	0	4	7	0	0	0	0	2
moderate	0	0	0	0	13	0	0	0	0	0
Yellowish material in lumen of proximal convoluted tubule										
minimal	0	0	0	0	13	0	0	0	0	0
Intracytoplasmic eosinophilic droplets in proximal convoluted tubules										
minimal	0	0	0	0	6	0	0	0	0	0
Total Examined	20	20	20	20	20	19	20	20	19	19

Incidence of Abnormalities in Kidney Pigmentation at 17 week Examination[2]

	males					females				
Dosage	0	10	30	60	360	0	10	30	60	360
Yellowish discolored cells of proximal convoluted tubule										
traces	0	3	1	0	4	0	0	0	0	0
minimal	0	0	0	5	0	0	0	0	0	0
Granular/clumped pigment in straight and/or proximal convoluted tubule										
traces	0	0	2	5	0	0	0	0	2	5
minimal	0	0	2	0	1	0	0	0	0	0
moderate	0	0	0	0	4	0	0	0	0	0
Total examined	5	5	5	5	5	5	5	5	5	5

1. From Tables A and C, Test Report HST 230, pp 23, 25.
2. From Tables B and D, Test Report HST 230, pp 24, 25.

Table 9

Incidence of Other Kidney Abnormalities at 13 Week Examination[1]

	males					females				
	0	10	30	60	360	0	10	30	60	360
Cortical areas of tubular basophilia	0	1	2	1	6	0	0	0	0	0
A cortical area of tubular basophilia	0	0	2	5	7	0	1	0	0	1
Total examined	20	20	20	20	20	19	20	20	19	19

Kidney Abnormalities Persisting at 17 Week Examination[2]

Cortical areas of tubular basophilia	0	0	0	1	1	0	0	0	0	1
A cortical area of tubular basophilia	0	0	0	0	1	0	0	0	1	0
Total examined	5	5	5	5	5	5	5	5	5	5

1. From Table 16, Test Report HST 230, pp. 68-75.
2. From Table 17, Test Report HST 230, pp. 76-83.