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

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MAY - 9 1989

MEMORANDUM

SUBJECT: 30-day Rat Study on Endosulfan

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: George LaRocca, PM-15
Registration Division, H7505C

FROM: Marcia Van Gemert, Ph.D.
Acting Chief, HFAS Branch
Health Effects Division H7509C

THRU: William Burnam
Deputy Division Director, HED, H7509C

Chemical: Endosulfan

Caswell No: 430

Hoechst Celanese has submitted a 30-day feeding study in rats in response to a registration standard. This study was reviewed by the contractor Dynamac, and the DER is attached.

Conclusions:
When endosulfan was fed to male Wistar rats for 30 days in the diet at levels of 360 or 720 ppm, there were no overt signs of toxicity or dose-related effects on body weight, food or water consumption, clinical observations, or ophthalmology. Two dosed animals died during the study with no discernible signs of intoxication. Absolute and relative liver weights of males receiving 360 and 720 ppm and kidney weights of males receiving 720 ppm were increased following the dosing period; organ weights of dosed males were similar to controls following a 30-day recovery period. Macroscopic examination revealed discoloration of the kidneys of dosed animals following the dosing period; histologically, the number and size of the lysosomes of the proximal convoluted tubules of the kidneys were increased in these animals following the dosing period with this finding exhibited to a greater extent in high-dose males. The renal changes were found to be reversible following the recovery period without evidence of renal lesions. There was no evidence of comparable lysosomal activity in the brain or liver. Electron microscopy and tissue residue analysis confirmed that a-endosulfan and to a lesser extent b-endosulfan, endosulfan sulfate, and endosulfan-lactone were stored temporarily in the kidneys during the dosing period; only negligible amounts of endosulfan metabolites were found in the liver. Based on kidney changes during the dosing period, the LOEL is 360 ppm, the lowest dose tested.

Classification: Core Supplementary (study of insufficient duration)

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NATIONAL SECURITY INFORMATION

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EPA: 68D80056
DYNAMAC No. 134-A
November 14, 1988

DATA EVALUATION RECORD

ENDOSULFAN

30-Day Feeding Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: William L. McLellan (for)

Date: 11/14/88

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EPA: 68D80056
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DATA EVALUATION RECORD

ENDOSULFAN

30-Day Feeding Study in Rats

REVIEWED BY:

Margaret E. Brower, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Margaret E Brower
Date: November 14, 1988

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L McLellan
Date: Nov. 14, 1988

APPROVED BY:

I. Cecil Felkner, Ph.D.
Subchronic and Chronic
Toxicology Studies
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Dynamac Corporation

Signature: I Cecil Felkner
Date: 11-14-88

Marcia Van Gemert, Ph.D.,
D.A.B.T.
EPA Reviewer and Section
Head, Section II
Toxicology Branch II (TS-769C)

Signature: Marcia Van Gemert
Date: 11/22/88

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DATA EVALUATION RECORD

STUDY TYPE: 30-Day feeding study in rats.

ACCESSION/MRID NUMBER: 407676-01.

TEST MATERIAL: Endosulfan; 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide.

SYNONYM(S): Thiodan; benzoepin.

STUDY NUMBER(S): 84.0585; report No. 87.0129.

SPONSOR: Hoechst Celanese Corporation, Somerville, NJ.

TESTING FACILITY: Hoechst AG, Pharma Research Toxicology and Pathology, West Germany.

TITLE OF REPORT: Endosulfan - active ingredient technical (code: HOE 002671 OI ZD97 000 3) 30-Day Feeding Study in Adult Male Wistar Rats.

AUTHOR(S): Leist, K. H. and Mayer, D.

REPORT ISSUED: November 15, 1984.

CONCLUSIONS: When endosulfan was fed to male Wistar Rats for 30 days in the diet at levels of 360 or 720 ppm, there were no overt signs of toxicity or dose-related effects on body weight, food or water consumption, clinical observations, or ophthalmology. Two dosed animals died during the study with no discernible signs of intoxication. Absolute and relative liver weights of males receiving 360 and 720 ppm and kidney weights of males receiving 720 ppm were increased following the dosing period; organ weights of dosed males were similar to controls following a 30-day recovery period. Macroscopic examination revealed discoloration of the kidneys of dosed animals following the dosing period; histologically, the number and size of the lysosomes of the proximal convoluted tubules of the kidneys were increased in these animals following the dosing period with this finding exhibited to a greater extent in high-dose males. The renal changes were found to be reversible following the recovery period without evidence of renal lesions. There was no evidence of comparable lysosomal activity in the brain or liver. Electron microscopy and tissue residue analysis confirmed that α-endosulfan and to a lesser extent β-endosulfan, endosulfan sulfate, and endosulfan-lactone were stored temporarily in the kidneys during the dosing period; only negligible amounts of endosulfan metabolites were found in the liver. Based on kidney changes during the dosing period, the LOEL is 360 ppm, the lowest dose tested.

Classification: Core Supplementary - study of insufficient duration according to EPA Pesticide Assessment Guidelines for subchronic studies.

A. MATERIALS:

1. Test Compound: Endosulfan, active ingredient technical; description: solid brown flakes; Code: HOE 002671 OI ZD97 000 3; purity: 97.9%.

2. Test Animals: Species: Wistar rat; strain: HOE: wiskf (SPF71); age: not specified; weight: males--138-168 g at study initiation; source: Hoechst AG, Pharma Research Toxicology, Kastengrund, SPF breeding colony.

B. STUDY DESIGN:

1. Animal Assignment:
Male rats were assigned to the following test groups (the period of acclimation was not reported) with a computerized randomization procedure:

Test group	Dose in diet (ppm)	Main study (4 weeks)	Recovery (8 weeks)[a]
1 Control	0	10	10
2 Low (LDT)	360	50	50
3 High (HDT)	720	50	50

[a]Recovery animals received test diets for 4 weeks followed by 4 weeks of diets without test material.

2. Diet Preparation: One-kilogram premixes containing 60 times the final dietary concentration of the test material were mixed into the basal diet (59 kg) biweekly. Samples were taken from each diet mix for concentration, homogeneity, and stability analyses. Storage conditions of the prepared diet were not reported.

Results: The diets were found to be homogenous (93-97%) and 84-99% stable over 14 days of storage. The test substance was reported to remain stable in the diet for more than 28 days. Recovery values of the diets ranged from 91 to 94% of the nominal values for the 360 ppm diet and 100 to 93% for the 720 ppm diet.

3. Food and Water Consumption: Animals received food [Altromin 1321 rat diet (Altromin Gmbh)] and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data. Body weights and absolute and relative organ weights were analyzed using the method of Sidak for parametric analysis and the method of Nemenyi/Sidak for nonparametric analysis.

5. Quality Assurance: A quality assurance statement was signed and dated March 27, 1987.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily 5 days per week and once daily on weekdays and holidays for signs of morbidity and mortality. Weekly examinations were conducted for neurological disturbances, opacity of the refracting media of the eyes, impairment of dental growth, and damage to the oral mucosa.

   Results: One male receiving 720 ppm died on study day 8 and one male receiving 360 ppm died on study day 29. These animals were reported to have died without any discernible signs of intoxication; no further information was provided. No compound-related clinical observations were reported.

2. Body Weight: Rats were weighed at study initiation and weekly thereafter to study termination.

   Results: Body weight gains were similar in dosed and control males.

3. Food Consumption and Compound Intake: Food consumption was determined and mean daily diet consumption was calculated at the same intervals as weighings. Efficiency and compound intake were calculated from the consumption and body weight gain data. Water consumption was determined once weekly.

   Results: Food and water consumption were similar in dosed and control rats. The mean compound consumption of rats receiving 360 and 720 ppm endosulfan over 30 days was 34 and 67.8 mg/kg/day, respectively.

4. Ophthalmological Examinations: Examinations of the opacity of the refracting media of the eyes were conducted weekly.

   Results: It was reported that there were no compound-related ophthalmological effects. However, neither data nor a statement signed by a certified veterinary ophthalmologist were present.

5. Hematology and clinical chemistry: These analyses were not performed.

6. Urinalyses: Urinalyses were not performed.

7. Sacrifice and Pathology: All animals that died and six rats/dose group that were sacrificed on schedule were subject to gross pathological examinations. These examinations included skin, orifices, eyes, teeth, oral mucosa, and internal organs.

   Following each scheduled sacrifice, livers, kidneys, and brains of all animals were removed and weighed. The left kidney and a section of liver from six rats/group were preserved in formaldehyde; the right kidney, a section of liver, and the brain (cerebrum, cerebellum, and brain stem) from these animals were preserved in Carnoy's fluid. Liver and kidney tissues from two rats/group were processed for electron microscopy; after staining with uranyl acetate, and lead acetate, thin and semi-thin sections were examined. All other liver and kidney tissue as well as the blood from all test animals were frozen in liquid nitrogen for tissue residue identification.

   Results:

   a. Organ Weights: The mean absolute and relative liver weights of males receiving 360 and 720 ppm endosulfan for 30 days, as well as the absolute and relative kidney weights of high-dose rats, were found to be slightly but significantly increased ($p<0.05$) (Table 1). The slight increase in relative brain weights of rats receiving 720 ppm was not considered to be of any toxicological importance. The organ weights of all dosed rats were found to be similar to the controls following the 4-week recovery period.

   b. Gross Pathology: Macroscopic examination of the kidney tissues of rats fed 360 or 720 mg/kg endosulfan for 30 days revealed a brownish discoloration not apparent in controls. The discoloration was apparent in all dosed rats selected for histologic examination (six/dose group). Dosed animals examined from the recovery group 30 days following dosing did not exhibit this effect.

TABLE 1. Body Weights and Selected Absolute and Relative Organ Weights (±S.D.) of Male Rats Fed Endosulfan for 30 Days

Dose Group (ppm)	Body Weight[a]	Liver		Kidney		Brain	
		Absolute (g)	Relative (%)	Absolute[b] (g)	Relative (%)	Absolute (g)	Relative (%)
0	317 ± 26(10)	12.29 ± 1.26	3.87 ± 0.16	1.96 ± 0.07(4)	0.61 ± 0.046	1.96 ± 0.05	0.62 ± 0.048
360	307 ± 20(49)	13.53 ± 1.43*	4.40 ± 0.30*	2.19 ± 0.20(43)	0.71 ± 0.045	1.95 ± 0.11	0.64 ± 0.043
720	294 ± 26(50)*	14.42 ± 1.65*	4.92 ± 0.43*	2.24 ± 0.21(45)*	0.77 ± 0.071*	1.98 ± 0.10	0.68 ± 0.66*

[a]Parentheses equal number of animals in control and dosed groups in which body weights and liver and brain weights were evaluated.

[b]Parentheses equal number of animals in control and dose groups in which kidney weights were recorded.

*Significantly different from controls at $p < 0.05$.

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c. Tissue Residue Determinations: Pooled tissues of 10 animals/group (liver, kidneys, and blood) were homogenized and extracted with acetone followed by aqueous methanol and dichloromethane. Aliquots of the extracts were analyzed by high-performance liquid chromatography and gas chromatography.

Results: The tissue residue levels of endosulfan and endosulfan metabolites following the 30-day dosing period are presented in Table 2; the tissue residue levels following the 30-day recovery period are presented in Table 3. Following dosing, α-endosulfan was found to be principally stored in the kidneys with the concentration stored in proportion to the dose level. The concentration of β-endosulfan was found to be 213 to 240 times less than that of α-endosulfan. In addition to α-endosulfan, only endosulfan-sulfate and endosulfan-lactone were detected to any appreciable extent in the kidneys. Endosulfan-sulfate (0.41-0.48 mg/kg) and endosulfan-lactone (0.45 - 0.86 mg/kg) were the major metabolites found in the liver. Only negligible amounts of endosulfan-lactone (0.04-0.06 mg/kg) and endosulfan-sulfate (0.01-0.02 mg/kg) were found in the blood. Following the 30-day recovery period, only traces of α-endosulfan (0.02 - 0.06 mg/kg) were found in the kidneys, indicating reversible storage.

The study authors reported that these findings had been noted previously in a 14-day feeding study[1] with α- and β-endosulfan at a dose level of 5 mg/kg in which the highest residues were found in the kidneys and elimination took place within a half-life of 7-10 days.

C. Microscopic Pathology:

1) Nonneoplastic: Following 30 days of dosing with the test compound, kidneys of animals receiving 360 and 720 ppm exhibited an increase in the number and size of lysosomes of some of the proximal convoluted tubules; this finding was reported to be exhibited to a greater extent in high-dose animals. Following 30 days of recovery, the renal changes appeared to recede and the microscopic appearance of the kidneys of dosed animals appeared to be similar.

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[1]Dorough et. al. Pest. Biochem. Physiol. 8(1978):241-252.

TABLE 2. Residue Levels of Endosulfan and Metabolites (±S.D.) in Rats Dosed for 30 Days

Metabolite	Endosulfan dosage (ppm)	Liver (mg/kg)	Kidneys (mg/kg)	Blood (mg/kg)
α-Endosulfan	360	0.025 ± 0.007(14/2)[a]	12.4 ± 4.8 (44/5)[a]	<0.01 (10/1)[a]
	720	0.013 ± 0.01(20/2)[a]	28.8 ± 12.1 (44/5)[a]	<0.01 (10/1)[a]
β-Endosulfan	360	< 0.01	0.058 ± 0.03	< 0.01
	720	< 0.01	0.12 ± 0.05	< 0.01
Endosulfan-sulfate	360	0.41 ± 0.04	1.24 ± 0.23	0.02
	720	0.48 ± 0.06	2.1 ± 1.1	0.01
Endosulfan-diol	360	< 0.01	0.02 ± 0.01	< 0.01
	720	< 0.01	0.06 ± 0.02	< 0.01
Endosulfan-hydroxyether	360	< 0.01	< 0.02	< 0.01
	720	< 0.01	< 0.02	< 0.01
Endosulfan-lactone	360	0.45 ± 0.04	1.34 ± 0.3	0.04
	720	0.36 ± 0.4	2.48 ± 0.7	0.06

[a]Number in parentheses indicates number of animals/number of samples per animal measured for liver, kidney, and blood analyses.

TABLE 3. Residue Levels of Endosulfan and Metabolites (±S.D.) in Rats Following a 30-Day Feeding Period and a Subsequent 30-Day Recovery Period

Metabolite	Endosulfan dosage (ppm)	Liver (mg/kg)	Kidneys (mg/kg)	Blood (mg/kg)
α-Endosulfan	360	< 0.01 (30/3)[a]	0.02 ± 0.01 (14/2)[a]	<0.01 (10/1)[a]
	720	< 0.01 (20/3)[a]	0.06 ± 0.05 (13/2)[a]	<0.01 (10/1)[a]
β-Endosulfan	360	< 0.01	< 0.02	< 0.01
	720	< 0.01	< 0.02	< 0.01
Endosulfan-sulfate	360	< 0.02	< 0.02	< 0.01
	720	< 0.02	0.03 ± 0.03	< 0.01
Endosulfan-diol	360	0.01 ± 0.01	< 0.02	< 0.01
	720	< 0.01	< 0.02	< 0.01
Endosulfan-hydroxyether	360	0.01 ± 0.01	< 0.02	< 0.01
	720	< 0.01	< 0.02	< 0.01
Endosulfan-lactone	360	0.04 ± 0.01	< 0.02	0.01
	720	0.03 ± 0.01	< 0.02	0.01

[a]Number in parentheses indicates number of animals/number of samples per animal measured for liver, kidney, and blood analyses.

There was reported to be no evidence of necrotic nor necrobiotic alteration of the renal cells. The lysosomal alterations were also observed by electron microscopy; lysosomal size only rarely exceeded the historical control limit of 3 µm and no essential difference existed in lysosomal content between dosed and control animals. Isolated lysosomal structures, up to 3.5 µm in size, were observed by electron microscopy in dosed animals prior to and following recovery; however, these findings were rare. The authors considered these isolated lysosomal findings to suggest that the test compound or metabolites were stored in the lysosomes and were degraded over time without cellular disruption. There was reported to be no evidence of comparable lysosomal activity in the brain or liver. Examinations of the liver revealed no abnormal changes in the parenchyma nor Kupffer cells.

The kidneys, liver, and brain of the two males that died during the study were in advanced stages of autolysis, and histolcgical results could not be detected.

D. STUDY AUTHORS' CONCLUSIONS:

Dietary administration of endosulfan to male Wistar rats for 30 days at concentrations of 360 or 720 ppm produced an increase in the size and number of lysosomes in the cells of the proximal convoluted tubules of the kidneys. These findings were found to be reversible following a 30-day recovery period. Examination by electron microscopy indicated that endosulfan or its metabolites were stored in the lysosomes of the kidneys and were degraded or excreted. Residue analysis confirmed that α-endosulfan and to a lesser extent ß-endosulfan were stored temporarily in the kidneys without resulting renal lesion. The NOEL is 720 ppm, equivalent to a daily test substance intake of approximately 57.8 mg/kg/day.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate although there were some deficiencies in the conduct of the study and in data reporting. Histological examination was performed on the kidney, liver, and brain of only 5/50 animals from the main and recovery groups. In addition, examination by electron microscopy was limited to only random sampling. Histological examinations must be conducted on more than 12% of the animals/group.

The number of samples collected for tissue residue analysis varied with each tissue and time of analysis. No explanation was reported for this variance.

Results reported (p. 276 of the study report) from the analytical laboratory indicated that α-endosulfan was retained only in the liver following the dosing period. According to the tabulated data and the study report summary, this was incorrectly reported. α-Endosulfan was retained primarily in the kidney following the dosing period. In addition to liver, kidney, and blood, the residue determination of adipose tissue of dosed animals would have provided additional data regarding residual effects of this chlorinated pesticide.

We agree with the study authors that the increased kidney weights were a reflection of the storage of endosulfan metabolites while the increased liver weights were a reflection of increased metabolic activity. However, we disagree with the NOEL of 720 ppm reported by the study authors based on recovery of the animals after the 4-week recovery period. The LOEL and NOEL should be based on compound-related changes experienced during the dosing period. Based on kidney changes during the dosing period, the LOEL is 360 ppm, the lowest dose tested.