US EPA ARCHIVE DOCUMENT

BC-446
TR-4892

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

004892

OFFICE OF
PESTICIDES AND TOXIC SUBSTANC

MEMORANDUM

SUBJECT: Review of Subchronic Oral Rat Study (30-day) Using Endosulfan -Technical

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

FROM: Margaret L. Jones Margaret L Jones 1/14/86
Review Section III
Toxicology Branch
HED (TS-769)

THROUGH: Clint Skinner, Ph.D., Head C. Skinner 1-15-86
Review Section III
and
Theodore M. Farber, Ph.D., D.A.B.T., Chief 1/20/86
Toxicology Branch

Compound: Endosulfan Technical Tox. Chem. 420

Registration No.: 154112 Registrant: American Hoechst

Accession No.: 257932 Tox. Project No.: 491

Action Requested: Review of the 30-day oral rat study using Endosulfan-Technical. This study is supplementary and does not fulfill any data requirements, but provides information which can be used with other data required to demonstrate the safety of Endosulfan.

Background: The present study was undertaken to examine the kidney effects which were found in the two-generation reproduction study and the 13-week feeding study. The study used two fairly high concentrations of test compound: 360 and 720 ppm. As described in detail in the attached Data Evaluation Report, the study looked at the effect of administration of the test compound for four weeks in one group and at the effect of a four week administration of the compound followed by an additional four week withdrawal period in another group. The study planned a pathology evaluation of the kidney lesions in order to identify their etiology.

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Conclusions: There was no NOEL for the kidney discoloration observed after four weeks of administration of test compound.

Absolute and relative liver weights were increased at both 360 and 720 ppm, the increases were significantly different from controls at 720 ppm. Kidney and brain weights were significantly increased at 720 ppm. The ratio of liver weight to brain weight was increased at 360 and at 720 ppm after the treatment period.

Enlargement and proliferation of lysosomes and granular pigmentation in the cells of the proximal convoluted tubules of the kidney were seen at 360 and at 720 ppm.

The effects described above were apparently reversible after the four week recovery period. After four weeks of administration of the test substance and four additional weeks for recovery, kidney discoloration disappeared, kidney, liver, and brain weights were normal, and proliferation and enlargement of lysosomes in the kidney cells of the proximal convoluted tubules apparently disappeared.

The study did not succeed in identifying the chemical nature of the granular substance observed in these cells. The exact cause of the kidney lesions remains unexplained.

The study is classified as Supplementary.

Reviewed by: Margaret L. Jones Margaret L. Jones 1/14/86
Section III, Tox. Branch, (TS-769)

Secondary reviewer: Clint Skinner, Ph.D. Clint Skinner 1-15-86 004892
Section III, Tox. Branch, (TS-769)

DATA EVALUATION REPORT

Study Type: Subchronic Oral Rat (30-day) Tox. Chem: 420

Accession Number: 257932

Test Material: Endosulfan - Technical

Synonyms: Thiodan, Thionex

Study Number: A 30776; 84.0585; (Translation Report No.: 85.0304); Project Tx 149/06/01 of 6 August 1984; Doc. No. 746

Registrant: American Hoechst

Sponsor: Pflanzenschutzforschung Biologie for Hoechst

Testing Facility and Archive: Pharma Forschung Toxikologie
Hoechst Aktiengesellschaft
Postfach 80 03 20
6230 Frankfurt Main 80

Title of Report: Endosulfan - active ingredient technical (Code: HOE 002671 OI ZD 97 0003) 30-day feeding study in adult male Wistar rats

Authors: Leist, Dr., Kramer, Prof., Kief. Prof., et.al.

Report Issued: March 22, 1985

Conslusions: There was no NOEL for the kidney discoloration observed after four weeks of administration of test compound. Absolute and relative liver weights were increased at both 360 and 720 ppm, the increases were significantly different from controls at 720 ppm. Kidney and brain weights were significantly increased at 720 ppm. The ratio of liver weight to brain weight was increased at 360 and at 720 ppm after the treatment period. Enlargement and proliferation of lysosomes and granular pigmentation in the cells of the proximal convoluted tubules of the kidney were seen at 360 and at 720 ppm.

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Classification: Supplementary

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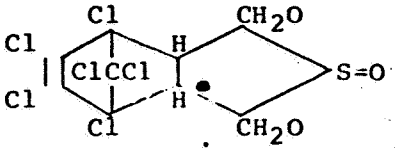
A. Materials:



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; Description: solid brown flakes, Batch No.: HOE 002671 OI ZD 97 0003; Analysis Certificate No.: 02582 (August 29, 1984) by Dr. Gorbach of Pflanzenschutz Analytik; 97.9% pure; Contaminants: not listed.

Test Animals: Species: 220 male Wistar rats, Strain: HOE:WISKf (SPF 71); Age: not indicated; Weight: 138-168 g; (x=153 ± 7 g) at start of preliminary study period (which lasted 5 days); Source: Hoechst AG, Pharma Forschung Toxikologie, Kastengrund, SPF-bred.

B. Study Design:

1. Animal Assignment: 220 male Wistar rats were assigned randomly to the following test groups:

Test Group (ppm)	(mg/kg/day)[1]	Main Groups	Recovery Groups
0	0	10	10
360	34.0	50	50
720	67.8	50	50

1. See discussion point 1 for information about the units used in the test report. The method used to convert ppm to mg/kg is not described in the test report.

Main group animals were killed after 4 weeks of treatment and the recovery groups were killed after the 4 week recovery period following the treatment period.

Animals were identified with fur markings ($KMnO_4$) and numbered ear tabs and assigned in pairs to wire mesh cages.

2. Diet Preparation: Diet was prepared twice during the test period, on 9/12/84 and on 9/26/84. Samples were analyzed for homogeneity and content of active ingredient in the batch on days 0, 7, and 14 after preparation. No information was given on how the prepared diet was stored during the two-week period.

Results: Two samples of each preparation were taken on days 0, 7, and 14. Content of the test substance in the prepared diet ranged from 274 to 376 ppm in the samples analyzed over the 14 day period starting 9/12/84 and from 591 to 722 ppm for the 720 ppm group. Since the test substance was 97.9% pure, the amount of active ingredient is somewhat less than these values. For the samples analyzed on 9/26/84 (2 samples of each preparation were taken on the day of preparation) the 360 ppm group ranged from 299 to 310 ppm and the 720 ppm group ranged from 550 to 707 ppm.

3. Animals received food (Altromin 1321 rat diet) and tap water ad libitum.

4. Statistics: Body weights, absolute organ weight, and relative organ weight were analyzed for significance at p= 0.05. No reference was given for the method employed.

5. Quality Assurance Statement: Dated 3/22/85; 5 inspections and 5 reports from 9/6/84 through 3/28/85 were examined and signed by the Study Director, S.J. Harston.

C. Methods and Results:

1. Observations: Animals were examined for behavior, general health conditions, body weights, and food and water consumption, during the test period.

Results: During the test period none of the animals showed any visible reactions to treatment. Two animals died: one in the 360 ppm group on day 29 and one in the 720 ppm group on day 8 of the study.

Body weight gains were similar to controls and food and water consumption of the test groups also paralleled that of controls.

2. Autopsy and Macroscopic Examination: Liver, kidneys, and brain were removed. Skin, orifices, eyes, teeth, oral mucosa, and internal organs were examined.

Results: After 30 days of treatment kidneys were found with brownish discoloration in both 360 ppm and 720 ppm dose groups. After the recovery period, kidney color was normal.

3. Organ Weights: Absolute and relative weights of liver, kidneys, and brain were measured.

Results: Absolute and relative liver weights were increased at both 360 and 720 ppm. Kidney and brain weights were significantly increased as compared to controls at 720 ppm. After the 4-week recovery period, no such differences were observed. The ratio of liver weight to brain weight was increased at 360 and at 720 ppm for the treatment group (without 30-day recovery period). This ratio was not increased at the end of the four week recovery period.

4. Microscopic Examination: Pathology examination included 6 animals from each group: controls, 360 ppm, 720 ppm, controls + 30-day recovery, 360 ppm + recovery, 720 ppm + recovery, and two animals dying on test.

Results: At 30 days, proliferation and enlargement of lysosomes and granular pigmentation were seen at 360 and at 720 ppm in the kidney in the cells of the proximal convoluted tubules. The finding was more pronounced at 720 ppm. The lysosomal changes in the proximal convoluted tubules regressed by the end of the 30 day recovery period. No lysosomal changes were seen in brain or liver. There was no evidence of necrosis in these cells at 30

days or at the end of the 30 days plus the additional 30 day recovery period according to the pathologist's report.

5. Identification of Residues in Tissues: Sections of liver, brain, and kidney were deep-frozen in liquid nitrogen and sent for analysis. The report states "The organs of 10 or 4 animals each were accumulated."[1] This statement is ambiguous and does not indicate exactly how many animals from each group were examined. Another statement is also misleading: "The blood of all animals was collected in fractions of 10 animals..."[2] Blood was collected and frozen for analysis but exactly which animals were tested is not clear.

Results: The attempt to identify residues found in tissues was unsuccessful due to "extreme technical difficulties".[3]

1. p. 15, Hoechst Test Report A 30776.
2. Ibid.
3. p. 17, Hoechst Test Report A 30776.

6. Statistical Analysis: Statistical analysis was performed on body weights, absolute organ weights, and relative organ weights using the methods of "Sidak" and "Nemenyi/Sidak". No references were given for the methods used.

Results: Significant differences were found ($p<0.05$) between body weight of controls and both dose groups: 360 ppm and 720 ppm, between pretest day 4 and day 30 of the study. There were no significant differences during or after the 30 day recovery period. Significant differences in relative organ weights were found at day 30 for liver at 360 and 720 ppm, and for kidneys at 720 ppm. No significant differences were noted after the 30 day recovery period.

Discussion: This study is Supplementary since the design is not included in the 1982 Pesticide Assessment Guidelines: Subdivision F for Hazard Evaluation (EPA-540/9-82-025). The information is nevertheless useful to consider along with other studies which fulfill Guideline requirements. Several areas of the study require further clarification before this information can be properly used with other data on this chemical:

1. The study uses "ppm" and "mg/kg diet" interchangeably. For the purposes of clarity, this review uses "ppm" rather than mg/kg since this term requires conversion using the factor 10. (For young rats, 1 mg/kg/day equals 10 ppm of diet.) The study should be corrected to indicate precisely which doses were tested and the correct conversion factors should be used if mg/kg will be discussed in the report.

2. Statistical methods were not properly referenced. For the methods to be properly evaluated, the references are necessary.

3. No hematology values were taken in the analysis of results. Yet, the study concludes that the granular deposits and the lysosomal proliferation seen in the cells of the proximal convoluted tubules of the kidney were due to breakdown and elimination of the test substance from the body. This conclusion is premature since the study does not explore other possible reasons for these deposits.