CASWELL FILE 420

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

009673

AUG 18 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Endosulfan - Submission of Supplemental Data for the 21-Day Inhalation Study.

TO: L Schnaubelt/R Richards
Product Manager/PM Team Reviewer (72)
Reregistration Branch, SRRD (H7508W)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 8/5/92
Toxicology Branch II, Section II
Health Effects Division (H7509C)

Thru: K. Clark Swentzel. K. Clark Swentzel 8/5/92
Toxicology Branch II, Head Section II
Health Effects Division (H7509C)

and

for Marcia van Gemert, Ph.D. James N. Rowe 8/10/92
Chief, Health Effects Division (H7509C)

Registrant:	Hoechst Celanese Corporation
Chemical:	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
Synonyms:	Endosulfan; Thiodan; Benzoepin; Endocide
Submission No.:	S417465
DP Barcode:	D177995
CASWELL:	420
MRID No.:	416675-00, 416675-01, 416675-02, 416675-03
Case/ID #:	819236/079401
Shaughnessy #:	079401
Action Requested:	Nothing specified.

Comment: The Registrant has submitted (1) an amendment (supplement MRID # 416675-01) to the original subchronic inhalation study report (MRID # 00147183), (2) a range-finding study report (MRID # 416675-02), and (3) comments (MRID # 416675-03) in response to the Agency's request for clarification/additional information regarding the subchronic (21-day) inhalation study (DER cover memo dated 7/20/86; Document # 005315).

BACKGROUND: The original TB review of the subchronic inhalation study listed several deficiencies in the study report, the main one being confusion as to the length of the exposure period (21 or 29

days). Additionally, there were no clear signs of toxicity observed at any dose level during the study.

(1) MRID # 416675-01: This is a supplement (entitled: Amendment to Doc. No. A29823 Report No. 84.0539, dated 15. August 1984) to the original report, and it was signed by a Quality Assurance (GLP) person who stated that "All changes made accurately reflect the raw data." In the original report, the discussion of the choice of dose levels indicated in one paragraph that 2 females died at the 0.0065 mg a.i./L dose level and in another paragraph that all animals survived at this dose level. Page 5 (6) of the supplement states that several typing errors occurred during translation of the German original report, and a re-evaluation caused some changes. Quoting from the supplement, " I. Introduction, page 7: It should read: All animals survived a mean concentration of 0.0024 mg Hoe 002671 active ingredient /l air (2ml/h of a 1% solution ... ." The statement continues and ends as follows: " an enhancement of intoxication and increase of lethalty (sic) could be expected following prolonged exposure period of 29 days."

The next page of the supplement then states that the II. Material and Methods, page 12: section of the report clearly indicated that 21 exposures during 29 days were performed.

Although the original report clearly indicated in several sections that 21 exposures were performed, the "typing errors" occurred in several sections also, **as well as in the current supplement,** submitted to rectify this situation. One cannot help but question where else such errors may have occurred in the report, especially in light of the fact that a Quality Assurance person supposedly reviewed this supplement.

(2) MRID # 416675-02: This is a final report of the range-finding study referred to in the subchronic study as the basis for the choice of dose levels. This study has been reviewed and the DER is attached. Under the conditions of the study, exposure to Endosulfan at dose levels of 0.0020 and 0.0065 mg/L via inhalation for 7 exposures in 9 days resulted in deaths of 2 of the 5 females at the high-dose level and negative body-weight gains in both sexes during the first few days of the study. The dose chosen as the high-dose for the 21-day inhalation study was 0.0020 mg/L, since increased toxicity was expected to occur following a longer period of exposure. The study (range-finding study) is classified Acceptable; it does not satisfy any guideline requirement, nor was it intended to do so.

(3) MRID # 416675-03: This is the Registrant's response to the Agency's review of the subchronic inhalation study. (a) With regard to the determination that no clear signs of toxicity were demonstrated during the study, the Registrant reiterates that in the range-finding study deaths occurred at the 0.0065 mg a.i./L level and impaired body weight gains were observed at the 0.0024 mg

a.i./L level after a 7-day exposure period. Since the subchronic study was of longer duration, 0.0020 mg a.i./L was chosen as the highest dose level. Additionally, the Registrant contends that Endosulfan displays a steep concentration-effect-relationship following inhalation exposures, although this is based on two levels only. Also submitted was a table listing the body-weight gains of the males following the exposure period, in which the 5 males of the recovery phase displayed a 9.9 to 22.6% lower gain than the controls. Although neither sex displayed any body weight effects during the exposure period and the number of recovery animals for each sex was only 5, the apparent effect suggests a possible delay in its manifestation. It is curious that the females did not demonstrate a similar effect, since the female is apparently more sensitive to the test material than the male.

(b) With regard to the length of administration of the test material, the Registrant reiterates that it is clearly stated in the report that there were 21 days of exposure. Although this is true, TB II points out that the original final report contained discrepancies in this aspect of the study to which clarification was required. It is ironic/frustrating that the clarification document (MRID # 416675-01, discussed above) contains the same discrepancy. (c) Concerning the monitoring of temperature and humidity during the study, the clarification submitted is adequate. (d) Historical control data for hematology and clinical chemistry parameters were requested, and these have been submitted. The data consist of two pages of columns of numbers, which are not clearly identified. Two columns are titled: Random sample, but it is not evident what that means (# of animals in sample ?). The data are not identified as to the species/strain of animal, the number of studies from which the data were obtained, when the studies were performed, and other basic information. NOTE: One column is labeled "Age group" and the following numbers appear: 0-7, 8-22, 23-45, 46-220, 221-450, 451-999. TB II assumes these are days, but is not aware of any studies which feature 999 day old rats. However, given the limitations of these data and the lack of a dose response for most of the parameters that showed statistically significant changes from the concurrent control values, with the exception of the decrease in leucocytes in the high-dose males, the decrease in calcium and increase in creatinine in the high-dose females, the differences observed may be discounted as due to treatment. The exceptions noted above indicate a possible kidney effect, and since other studies indicate the kidney as a target organ, these effects are considered treatment-related.

CONCLUSION

The subchronic (21-day) inhalation study can be upgraded to Core Minimum, based on the submitted data/information. The NOEL is set at 0.0010 mg a.i./L, the LEL at 0.0020 mg a.i./L, based on lower body-weight gain and leucocyte counts in the males and decreased calcium and increased creatinine values in the females. This study

satisfies the guideline requirement (82-4) for a subchronic inhalation toxicity study.

NOTE: The "bean sheet" indicates that this submission was received by the Agency on 10/24/90 and that it was "due out" 02/21/91. HED received it on 5/15/92. Had the data not been adequate, a new study would have been required. It has been nearly 2 years since this information was submitted to the Agency (NOT to TB II), which puts TB II in an awkward position were we to request a repeat of the study.

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 8/5/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 8/5/92
Section II Head, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 7-day inhalation - rat

TOX. CHEM. NO.: 420

MRID NO.: 416675-02

Shaughnessy No.: 079401

TEST MATERIAL: Endosulfan technical

SYNONYMS: Hoe 002671 OI ZD97 0003

STUDY NUMBER: A 44125; Report No. 90.1057

SPONSOR: Hoechst Celanese Corporation, Somerville, NJ

TESTING FACILITY: Pharma Research Toxicology and Pathology, Hoechst Aktiengesellschaft, Federal Republic of Germany

TITLE OF REPORT: SHORT REPORT Endosulfan - active ingredient technical [Code. Hoe 002671 OI ZD97 0003] Repeated dose inhalation toxicity [7 exposures in 9 days] in Wistar rats - Preliminary study to Report No. 84.0539

AUTHORS: R. Hack and K.-H. Leist

REPORT ISSUED: September 26, 1990

QUALITY ASSURANCE: A quality assurance statement was provided, which indicated that the final report of the study was inspected. It is not clear whether any inspections were performed during the conduct of the study.

CONCLUSIONS: Under the conditions of the study, exposure to Endosulfan at dose levels of 0.0020 and 0.0065 mg/L via inhalation for 7 exposures in 9 days resulted in deaths of 2 of the 5 females at the high-dose level and negative body-weight gains in both sexes during the first few days of the study. The dose chosen as the high-dose for the 21-day inhalation study was 0.0020 mg/L, since increased toxicity was expected to occur following a longer period of exposure.

Classification: Acceptable. This is a range-finding study; it does not satisfy any guideline requirement, nor was it intended to do so.

A. MATERIALS

1. Test Compound: Endosulfan technical; Description: brown flakes; Batch #: Certificate of analysis # 02184; Purity: 97.2%; Source: not stated.

2. Test Animals: Species: rat; Strain: Wistar Hoe : WISKf(SPF71); Age: ≈4-6 weeks; Weight: ♂♂-191 grams, ♀♀-186 grams; Source: Hoechst AG, Pharma Forschung Toxikologie, Kastengrund.

3. Statistics: Statistical procedures were not mentioned.

B. STUDY DESIGN

1. Methodology: Rats were randomly assigned (procedure not specified) to one of two concentrations of test material (5/sex/group). The rats were housed individually during exposure periods and in groups of 5 after exposure (apparently sexes separated) and were provided with food (Altromin 1324 pellets Altromin GmbH, Lage/Lippe) and tap water ad libitum during nonexposure periods. The rats were placed (individually) in cylindrical plastic tubes and exposed nose-only to Endosulfan (concentrations of either 0.0024 and 0.0065 mg test material/l air) for 6 hours per day, five days a week, with a total of 7 exposures over 9 days (not exposed on weekends). The volume applied was 2.0 mL/hour for the low dose and 5.0 mL/hour for the high dose. A 1% solution (vehicle: ethanol-polyethylene glycol 400 1:1) of the test material was prepared freshly each day.

2. Generation of Test Atmosphere and Exposure: Each exposure was conducted in an inhalation chamber, which consisted of a stainless-steel and glass cylinder with a volume of 80 L, standing in a vent pipe with a volume of ≈ 4 m^3. The chambers were operated under dynamic conditions. After passing through an oil separation filter and an absolute filter, air was pumped into a special nozzle with a supply pipe for the test material. The air supply at the nozzle was maintained at 800 L/hour. The 1% solution of test material was injected into the nozzle at a constant speed by means of a continuous infusion apparatus. The primary aerosol formation occurred in a 10-liter four-necked round-bottomed flask. Smaller aerosol particles (secondary aerosol) entered the inhalation chamber through a rising tube. The test atmosphere was drawn out of the inhalation chamber at a rate of 1100 L/h. It was stated that the difference in rate between the introduction of air at 1000 L/h and its extraction at 1100 L/h was necessary to maintain the kinetic energy of the aerosol particles and to ensure a largely laminar flow of the aerosol from top to bottom. The concentrations were measured gravimetrically by means of membrane filters 50 mm in diameter with a pore width of 0.65 µm (Sartorius Membranfilter GmbH, Göttingen). The air flow rate was 3 L/min., equivalent to an induction speed of 1.25 m/sec. Air samples were taken (in the respiratory region of the rats) for gravimetric analysis at 30-minute intervals after the start of exposure during each exposure period. Concentrations were determined on samples taken on day 1, 2, 6, and 8 of the study by gas chromatography with an ECD, with the readings being converted to give values for 97.2%

a.i.. The aerosol particle size distribution of the test material was measured using the Model 225 Particle Counting System manufactured by Kratel GmbH, Gerlingen 2. The mean percentage occurrence of the following equivalent diameters was determined. NOTE: It was stated that "Practicle (sic) size analysis was performed once and twice per hour, respectively.", which is confusing. No information was provided on whether chamber temperature and relative humidity were monitored during each exposure, and no mention was made that the rats were returned to their group housing following each exposure.

RESULTS

1. Analyses Gravimetric concentrations: The mean values were reported as ≈171 mg and 490 mg, corresponding to concentrations of 0.0024 mg and 0.0065 mg a.i. by chemical analysis. Chemical concentration: The concentration of a.i. in the respiratory air was determined to be 0.0024 and 0.0065 (units not reported in the table of results). Particle size analysis: The 0.0024 mg/L group had 91.8-97.5% of the particles with a diameter < 6μm and the 0.0065 mg/L group had 87.6-90.2% of the particles with a diameter of < 6μm. The data indicate that an adequate % of the particles at both concentrations were ≤ 1μm.

Particle Size Distribution (%)

Dose/days	0.0024 mg/L		0.0065 mg/L	
particle size (μm)	0.30-0.49	0.5-1.49	0.30-0.49	0.5-1.49
1	19.58	33.21	16.87	28.33
2	16.00	26.53	17.08	27.15
5	18.62	29.45	14.48	25.18
6	21.95	28.91	14.25	24.71
7	27.50	34.53	14.46	24.99
8	15.02	28.90	14.26	23.18
9	23.67	30.13	13.87	21.56

2. Clinical Observations: The behavior and general health of the rats was monitored during and after each exposure, and the rats were observed once a day on weekends. Body weights were recorded daily throughout the study.

RESULTS

Survival and Clinical Observations

There were 2 deaths at the high-dose level (♀♀ on day 8 of exposure) during the study; all other rats survived to termination. There were no clinical signs observed in the low-dose rats. Tremors, trembling, tonic-clonic convulsions, and reduced corneal reflexes were observed in the high-dose females, and both sexes displayed disequilibrium and irregular breathing.

Body Weight
The author stated that body weight gains were impaired in both sexes at both dose levels. However, the males at both dose levels all gained weight by study end (mean gain: low dose-12.4 grams; high dose-10.2 grams). A negative body-weight gain was displayed in both sexes through day 3. With regard to the females, 3 of the 5 low-dose rats showed lower body weights at day 9 compared to day 1 (mean gain of 0.6 grams). Of the 3 surviving high-dose females, one showed a lower weight at day 9 compared to day 1 (mean gain of 5.7 grams).

Body-Weight Gain (grams)

Interval (days)	MALES		FEMALES	
	low dose	high dose	low dose	high dose
1-9	12.4	10.2	0.6	5.7
1-2	-0.4	-3.4	-3.4	-6.8
1-3	-5.0	-7.0	-7.8	-6.6
1-4	5.2	3.8	-1.0	0.2
1-5	14.6	13.4	6.0	7.2

3. Sacrifice and Pathology

After the last exposure period, the rats were sacrificed, dissected, and the liver was weighed.

RESULTS

Gross Pathology: There were no reported gross pathological findings.

Organ Weights: There was no effect of treatment on liver weight.

C. DISCUSSION

This was a range-finding study. Two deaths occurred in the female group at the highest level (0.0065 mg/L) administered. Although there was an overall gain in body weight in both sexes, negative gains were displayed during the first 3-4 days of the study. Females appeared to be more sensitive to treatment than the males. Based on the results of this study, a dose of 0.0020 mg/L was chosen as the highest dose for the 21-day inhalation study.

D. CONCLUSION

Under the conditions of the study, exposure to Endosulfan at dose levels of 0.0020 and 0.0065 mg/L via inhalation for 7 exposures in 9 days resulted in deaths of 2 of the 5 females at the high-dose level

and negative body-weight gains in both sexes during the first few days of the study. The dose chosen as the high-dose for the 21-day inhalation study was 0.0020 mg/L, since increased toxicity was expected to occur following a longer period of exposure. This is a range-finding study, which is classified Acceptable. It does not satisfy any guideline requirement, nor was it intended to do so.

DISCREPANCIES IN THE FINAL REPORT.

Apparent "typos" were found on page 7, paragraph 3 (rawdata); page 12, first paragraph/last word under 4.5.2: both should read bath; page 12 first word of last sentence should read Particle, not Practicle; page 15, last sentence under 5.2.1: the word irregular should precede breathing; page 15, first paragraph under 6., the second word should be data, not date.