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

MAY 18 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTAI

MEMORANDUM

SUBJECT: Endosulfan: Review of Four Toxicology Studies and Three Dermal Absorption Studies

Caswell No. 420 HED Proj. No. 9-1300
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TO: G. LaRocca, PM (15)
Registration Division (H7505C)
and
K. Samek / B. Lowery, PM Team 74
Special Review and Re-registration Division (H7508C)

FROM: Whang Phang, Ph.D. 5/14/90
Pharmacologist
HFAS/Tox. Branch II/HED (H7509C)

THROUGH: K. Clark Swentzel, Section Head 5/14/
and
Marcia van Gemert, Ph.D. 5/14/90
Branch Chief
HFAS/Tox. Branch II/HED (H7509C)

The registrant, Hoechst Celanese, has submitted several toxicology studies at various times. Some of these studies have been reviewed by Dynamac Corp. and are approved by HFAS/Tox. Branch II. The evaluation of these studies have been requested by both the Registration Division and the Special Review and Re-registration Division at different times. The Data Evaluation Report (DER) of each study is attached, and the conclusions of these studies are presented below:

1) Ebert, E., Endosulfan-Water-Dispersible Powder (50%) (Code: Hoe 002671 OI WP50 A501) Subchronic Dermal Toxicity (21 Treatments in 30 Days) in Wistar Rats. Pharma Research Toxicology and Pathology, Hoechst AG. May 17, 1987. EPA MRID No. 410485-06.

Groups of Wistar rats (11/sex/dose) received endosulfan by dermal application for 30 days at doses of 0, 160, or 640 mg/kg (males) or 0, 80, or 160 mg/kg (females). Additional groups (6 rats/sex) received 40 mg/kg for the same duration. Following

the treatment period, groups of 5 rats/sex/dose were maintained without treatment for a 35-day recovery period. The results showed that 1 mid- and 3 high-dose females died during the study. There were no signs of neurological disturbance. Body weights and body weight gains of the high-dose (640 mg/kg) males were depressed from treatment day 10 to study termination, and the decrease in body weight continued during the recovery period. In females, treatment-related effects on body weight were not seen. Neither gross nor microscopic dermal changes were detected in the test animals. Following dosing, reticulocyte counts of mid- and high-dose males and level of cholesterol and total lipids in high-dose females were increased. In mid- and high-dose females, serum cholinesterase activity was decreased during treatment period and continued to be decreased during the recovery period. However, brain and erythrocyte cholinesterase activities were not affected by the compound. There were no treatment-related effects seen in organ weights. Compound-related histopathologic changes were not detected in the treated animals. Based on the body weight loss in males and mortality in females, the LEL for systemic toxicity was 640 mg/kg for males and 80 mg/kg for females; NOEL, 160 mg/kg for males and 40 mg/kg for females.

This study is classified as core minimum, and it satisfies the data requirements for a 21-day dermal toxicity study (Guideline No. 82-2).

It should be noted that in the Data Review Record the product manager, G. LaRocca, specifically requests the reviewer to comment on any possible findings of the kidney effects. Based upon the data presented, kidney effects were not found in either gross or microscopic examinations.

2). Thevanaz, P., et al., Subchronic (4-week) Repeated Dose Dermal Toxicity Study in Rats. Research and Consulting Co. AG. Study No. 094590; Oct. 4, 1988. EPA MRID No. 410485-05.

Groups of 15 Wistar rats/sex received endosulfan (emulsified concentrate) with dermal application at doses of 0, 27, 54, or 81 mg/kg for males and 0, 9, 12, 18, or 36 mg/kg for females for a total of 21-22 applications. Additional groups of 15 males and 15 females were treated with the formulation base in distilled water or the water (vehicle) in a similar fashion as the compound treated animals. Five rats/sex from each dose were designated as the recovery groups which were kept for an additional 4 weeks after the end of the treatment period. The results showed that there was an increase in the incidence of erythema in the endosulfan and formulation base treated animals, and this effect persisted through the first week of the recovery period. Clinical signs such as tremor, trismus, and extension spasms were seen in 81 mg/kg males and 12, 18, and 36 mg/kg females. Four 36 mg/kg females, one 18 mg/kg female, and one 12 mg/kg female died during

the study. Plasma cholinesterase activity of females which received 12, 18, and 36 mg/kg was decreased. Neither brain nor erythrocyte cholinesterase activity was affected by the treatment. No compound-related changes in organ weights were reported. In addition, no treatment-related increase in histopathologic changes was seen. Based upon the mortality and clinical signs indicative of the central nervous system effect, the LEL for systemic toxicity is 81 mg/kg in males and 12 mg/kg in females; NOEL, 54 mg/kg in males and 9 mg/kg in females.

This study is classified as core supplementary because the report does not present the incidence data for the treatment-related clinical signs of systemic toxicity. At the present, this study does not satisfy the data requirements for a 21-day dermal toxicity study (Guideline No. 83-2).

3). Brunk, R., Endosulfan-Substance Technical (Code: Hoe 002671 OI ZD96 0002) Testing for Toxicity by Repeated Oral Administration (1-Year Feeding Study) to Beagle Dogs. Hoechst Aktiengesellschaft Pharma Research, Toxicology and Pathology; Study No. 87.0643. Jan. 20, 1989. EPA MRID No. 410995-01.

Groups of Beagle dogs (6/sex/dose) received endosulfan at dietary concentrations of 0, 3, 10, and 30 ppm for a year. Additional groups (6/sex) were initially fed 30 ppm which was increased to 45 ppm after 54 days into the treatment; the concentration of the test material was further adjusted to be 60 ppm after 106 days. The animals at 60 ppm developed severe nervous symptoms which included a loss or weakening of placing and righting reactions. A marked weight loss was also observed in this dose level, and the surviving dogs of this group showed poor health and were sacrificed on days 146 or 147. At the 30 ppm level, both males and females showed signs of tonic contractions of the abdominal muscle and chaps a few hours after feeding. No other compound-related effects were reported. Based on the decreased weight gain in males and neurologic signs in both sexes, the LEL for chronic toxicity is 30 ppm (0.65 mg/kg); NOEL, 10 ppm (0.57 mg/kg).

This study is classified as core minimum, and it meets the data requirements for a chronic feeding study in non-rodent (Guideline No. 83-1).

4). Runkman, S. et al., Combined Chronic Toxicity/Carcinogenicity Study-104 Week Feeding in Rats. Huntingdon Research Centre Ltd.; Study No. HST 289/881067. April 1, 1989. EPA MRID No. 410995-02.

Groups of rats (50/sex/dose) received endosulfan at dietary concentrations of 0, 3.0, 7.5, 15.0, and 75.0 ppm for 24 months.

Satellite groups (20 rats/sex/dose) were also included in the study. In 75.0 ppm males and females, a significant ($p<0.01$) decrease in body weight gains was seen. In gross examination, an increase in the incidence of bilateral enlarged kidneys in 75.0 ppm males and females was reported, and this macroscopic observation in both males and females of 7.5 ppm was associated with an increase in the incidence of "marked progressive glomerulonephrosis". An increase in the incidence of aneurysms in the blood vessels was seen in the 75.0 ppm males. The tumor incidence was comparable between the treated and the control rats. Based upon these findings, the LEL was established as 75.0 ppm (3.45 mg/kg); NOEL, 15.0 ppm (0.65 mg/kg).

The dosage levels of endosulfan appear to be adequately tested in this study. The study is classified as core minimum, and it satisfies the data requirements for combined chronic feeding/ carcinogenicity study in rats (Guideline Nos. 83-1 & 83-2).

5). Lachman, G., Dermal Absorption of ^{14}C-endosulfan in Rhesus Monkeys. Battelle, FRG; Report No. BIEVV-66.697. May 8, 1987. EPA MRID No. 410485-03.

This study was evaluated by Dynamac Corp., and the Data Evaluation Report (DER) prepared by Dynamac Corp. along with the study were secondarily reviewed by Dr. Robert Zendzian of HED. The Tox. Branch II agrees with the conclusion derived by Zendzian that the study is unacceptable because of the deficiencies in dose application, animal handling, and the recovery of the radioactivity.

6). Craine, E. M., A Dermal Absorption Study in Rats with ^{14}C-endosulfan With Extended Test Duration. WIL Research Labs., Lab. Project No. WIL 39029. Nov. 17, 1988. EPA MRID No. 410485-04,

Female rats were dosed dermally at 1.9, 21.9, and 231.4 mg/cm^2, washed at 10 hours, and followed to 168 hour; 44.8, 46.4, and 20.3% of the respective doses were absorbed.

This study has been classified as acceptable, and it meets the data requirements for a dermal absorption study in rats (Guideline No. 85-2).

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

November 29, 1989

SUBJECT: Endosulfan, Dermal Absorption Study and DYNAMAC Review Thereof

TO: Whang Phang Ph.D.
Pharmacologist
Review Sec II
Toxicology Branch II, HED

FROM: Robert P. Zendzian Ph.D.
Senior Pharmacologist
Health Effects Division (H7509C)

At your request I have reviewed the following study and the DYNAMAC review thereof. The study is of no scientific or regulatory value. The review is unacceptable.

Dermal Absorption of ^{14}C-Endosulfan in Rhesus Monkeys, G. Lachman, Battelle-INstitut Toxikologie und Pharmakologie, Frankfurt, FRG, BIEV-V-66.697, 5/8/87, MRID 410485-03.

Data Evaluation Record, Endosulfan, Dermal Absorption Study in Monkeys. N.P. Hajjar & W.L. McLellan, EPA No. 68D80056, DYNAMAC No. 210-C, Task No. 2-10C, Nov 15, 1989.

The following deficiencies are noted in the study.

1. Dosing. Radiolabeled test compound was suspended in the Thiodan EC blank solution and applied with a paint brush to shaved skin of the neck and shoulders of the monkeys. Using this crude nonquantative method only 58 and 71% of the dose was applied per monkey for doses of 2.2 and 3 mg/kg. The area dosed was not given. Dermal doses for absorption studies MUST be on a mass/unit area (mg/cm^2) as the absorption rate depends critically on this parameter.

2. The dosed animals were exposed for 10 hours in metabolism cages. The application site was not protected nor were the animals restrained to prevent ingestion of test material or loss from falling or being rubbed off. Thus we do not know if the entire dose remained on the animals for the exposure period or if the material appearing in the urine was dermally absorbed, ingested or simply fell into the collection container.

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3. Total recovery of radioactivity was 47.76 and 54.58% of the administered dose. This is an unacceptably low recovery. This deficiency raises serious questions about the quantative analytical procedures used in the laboratory. Since an apparently complete balance determination was made the only conclusion that is possible is that the quantitation data are wrong.

There exists no scientific or regulatory rationale for using the primate for this kind of study. The Rhesus is a very valuable experimental animal which is large and hard to handle. It is not a minature human being and data obtained from the Rhesus are not necessarily directly applicable to man. The method of dose application, animal handling and recovery of test compound clearly show that the laboratory was unable to perform an acceptable study in a primate.

The DYNAMAC review notes some of the deficiencies in the study but fails to reach the necessary conclusion that the study is unacceptable and that the laboratory appears unable to perform or recognize an acceptable study.

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EPA No.: 68D80056
DYNAMAC No.: 210-C
TASK No.: 2-10C
November 15, 1989

DATA EVALUATION RECORD

ENDOSULFAN

Dermal Absorption Study in Rhesus Monkeys

STUDY IDENTIFICATION: Lachman, G. Dermal absorption of ^{14}C-endosulfan in Rhesus monkeys. (Unpublished report No. BIEV-V-66.697 performed by Battelle, Frankfurt, FRG, for Hoechst Celanese Corporation, Somerville, NJ; dated May 8, 1987.) MRID No. 410485-03.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____

Date: _____

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1. CHEMICAL: Endosulfan; 6,7,8,9,10,10-hexachloro-1,5,5a, 6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide, (alpha:beta isomer ratio of [redacted].

2. TEST MATERIAL: [5a, 9a-^{14}C]Endosulfan [redacted] mixture of alpha:beta isomers) with a specific activity of 26.9 mCi/g and a radiochemical purity of >98 percent was used.

3. STUDY/ACTION TYPE: Dermal absorption in Rhesus monkeys.

4. STUDY IDENTIFICATION: Lachman, G. Dermal absorption of ^{14}C-endosulfan in Rhesus monkeys. (Unpublished report No. BIEV-V-66.697 performed by Battelle, Frankfurt, FRG, for Hoechst Celanese Corporation, Somerville, NJ; dated May 8, 1987.) MRID No. 410485-03.

5. REVIEWED BY:

Nicolas P. Hajjar, Ph.D.
Principal Reviewer
Dynamac Corporation
Signature: _____
Date: _____

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation
Signature: _____
Date: _____

6. APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation
Signature: _____
Date: _____

K. Clark Swentzel, Ph.D.
EPA Reviewer, Section II
Toxicology Branch II
(H-7509C)
Signature: _____
Date: _____

Marcia Van Gemert, Ph.D.
Branch Chief, Section II
Toxicology Branch II
(H-7509C)
Signature: _____
Date: _____

7. CONCLUSIONS:

The dermal absorption of [5a, 9a-^{14}C]-endosulfan was studied in two male Rhesus monkeys following application doses of 2.2 or 3.0 mg/kg for a period of 10 hours. Radioactive residues in blood and plasma increased gradually with time after application and reached a plateau level by 24 to 36 hours post-application (25 to 40 ng/mL). Total recovery of radioactivity accounted for only 48 and 55 percent of the applied dose. Approximately 3 percent of the dose was eliminated in the urine and 4 to 5 percent in the feces. ^{14}C residues in tissues accounted for about 1.3 percent, and about 10 percent was recovered in the remaining carcass. ^{14}C residues in brain (8 ng/g) were lower than those found in blood, but ^{14}C residues for other tissues were higher ($\leq$1.7 μg/g). Endosulfan-diol and an unidentified metabolite were the major components in urine, whereas the same unidentified metabolite was the major component in feces.

Because of the low total recovery of radioactivity, this study provides only supplementary data.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. Dosing solutions were prepared by mixing 19 ng [^{14}C]-endosulfan in 50 μL thiodan EC blank solution. The resulting concentration of the test compound was 29.48 percent (w/w). Separate dosing solutions were prepared for each animal.

2. Two male Rhesus monkeys weighing 5.0 and 4.5 kg were obtained from Shamrock Farms, Sussex, U.K. The animals were kept in metabolism cages and acclimated to test conditions for 2 weeks prior to dosing.

3. The dosing solution was mixed with 2.5 mL of distilled water prior to application. The resulting suspension was sonicated; paint brushes were then immediately used to apply the suspension to the shaved skin of the necks and shoulders of the animals. The amount of radioactivity applied was determined by subtracting the radioactivity remaining from the total radioactivity present in each vial. Monkey 1 received a dose of 2.2 mg/kg, and monkey 2 received a dose of 3.0 mg/kg.

[1]Items appropriate to only this DER have been included.

4. Animals were placed in metabolism cages, and the treated skin was washed with a soap solution 10 hours postapplication. Blood samples (4 to 5 mL) were collected at various intervals postdosing, and total radioactivity in blood and plasma was assayed. Urine and feces were collected at 24-hour intervals. The cage was also rinsed with distilled water after 24 hours. Samples were radioassayed and stored at -20°C. Animals were killed 96 hours after dosing, and the following tissues were removed: liver, kidneys, brain, fat, and treated skin. The residual carcasses were then stored at -20°C.

   Owing to low recovery of radioactivity, the carcasses were thawed overnight 9 weeks later and the following tissues were removed: total muscle below the treated skin, muscle of the hind limb (to compare it with the muscle below the treated skin), skin at the inner side of the hind limbs, and skin at the back side of the hind limbs (to compare it with the skin of the inner side). The residual carcasses were separated into smaller portions for dissolution.

5. All samples were measured in duplicate. Blood, plasma, feces, and tissues were radioassayed by a liquid scintillation counter (LSC) following combustion in a Packard Tri-carb sample oxidizer. Various extraction procedures were used to extract radioactive residues in the various tissues. All values were corrected for combustion and counting efficiencies, and background values were subtracted. Dosing vials, paint brushes, and washcloths were rinsed, then combusted and radioassayed. Urine aliquots were radioassayed directly.

6. Feces were homogenized twice in acetonitrile/water (4/1; v/v), and the radioactivity in the homogenate and in the sediment and supernatant following centrifugation was assayed. Sediments were also transferred into dialysis bags, and residues were then dried and combusted prior to radioassay by LSC. The 0- to 24-hour urine samples and extracts of the 72- to 96-hour feces samples were further analyzed quantitatively by thin-layer chromatography (TLC). Samples were also incubated with glucuronidase and arylsulfatase, and hydrolyzed products were analyzed by TLC.

   Quantitative analyses of urinary metabolites from monkey 2 were conducted on urine samples collected 0 to 24, 24 to 48, and 48 to 72 hours postdosing. For feces, equal volumes of extracts from feces collected

from both animals at 24 to 48, 48 to 72, and 72 to 96 hours were pooled and analyzed. Samples were analyzed and quantified by high-performance liquid chromatography (HPLC).

B. Protocol: A protocol was not presented.

12. REPORTED RESULTS:

A. Blood and plasma radioactivity levels increased gradually following application of [^{14}C]endosulfan. A steady-state level was reached 24 to 36 hours postdosing (Table 1). ^{14}C residues in plasma were higher than those found in blood with a ratio of 0.65 (or 1-hematocrit). This ratio increased to 0.75 to 0.8 at 72 and 96 hours.

B. Total recovery of radioactivity accounted for 48 and 55 percent of the dose applied to monkey 1 and 2, respectively (Table 2). Approximately 3 percent of the dose was eliminated in the urine and 4 to 5 percent in the feces. ^{14}C residues in tissues accounted for about 1.3 percent of the dose, and about 10 percent was recovered in the remaining carcass.

Residues in brain (8 ng/g) were lower than those found in blood, whereas the other tissues contained higher ^{14}C residue levels (Table 3). Approximately 40 to 62 percent of the radioactivity in liver and kidney was extractable in organic solvents, whereas 85 percent of the radioactivity in fat was extractable.

C. TLC analyses of urine revealed the presence of endosulfan-diol, ß-endosulfan, and α-endosulfan, together with one unidentified metabolite and radioactivity remaining at the origin. No qualitative differences were noted following glucuronidase/arylsulfatase hydrolysis. Most of the radioactivity extracted from the feces was associated with the origin of the chromatogram even after enzyme hydrolysis. Low amounts of α- and ß-endosulfan and endosulfan-diol were detected.

Purification of metabolites was performed by HPLC. Tables 4 and 5 present the amounts of each metabolite detected in the urine and feces, respectively. Endosulfan-diol and an unidentified metabolite were the major components in urine. The amounts of the unidentified metabolite increased following incubation with glucuronidase with a corresponding decrease in the concentration of diol. Most of the radioactivity in feces was associated with the same unidentified metabolite. The unidentified metabolite was

Endosulfan toxicology review

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Pages 12 through 16 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

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___ The document is not responsive to the request

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thought to be an oxidation product of endosulfan. α- and ß-endosulfan were present in similar proportions as compared with a ratio of 7:3 found in the test material.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The dermal absorption of [^{14}C]endosulfan was studied with two male rhesus monkeys after 10-hour applications of 2.2 mg/kg for monkey 1 and 3.0 mg/kg for monkey 2.

Blood and plasma levels for monkeys 1 and 2 reached plateau values of 25 or 35 ng/mL after 36 hours, respectively (mean values of both animals). The excretion of [^{14}C]endosulfan equivalents amounted to 3.7 percent of the dose for urine and 4.3 percent for feces (mean values) at the end of the study (96 hours after application).

At the end of the experiment, 8.3 percent and 13.9 percent of the dose were detected in the treated skin of monkey 1 and monkey 2, respectively. The radioactivity of the residual carcasses amounted to 10.9 percent (monkey 1) and 10.2 percent (monkey 2). At the end of the experiment, a total recovery of radioactivity of 47.8 percent was obtained for monkey 1 and 54.6 percent for monkey 2 (including cloths and radioactivity in the cages).

The levels of [^{14}C]endosulfan equivalents in the brain were very low, whereas kidney, fat, and liver showed higher values. As compared to the respective blood levels, the values obtained for kidney, fat, or liver were 3-, 9-, or 19-fold greater. All (mean) values were below 0.5 ppm.

For urine, endosulfan-diol was the most important metabolite, the maximum amount of which (1.5 percent of the dose) was excreted between 0 and 24 hours. A second metabolite, x, presumably endosulfan hydroxycarboxylic acid, amounted to 0.6 percent of the dose for the same sampling interval. Only negligible amounts of α- and ß-endosulfan were found in the urine.

For feces, x was the most important metabolite (0.4 percent of the dose, 24- to 48-hour and 72- to 96-hour sampling intervals); endosulfan-diol could be detected only for the 24- to 48-hour sampling interval. The sum of α- and ß-endosulfan amounted to 0.1 percent of the dose for both the 24- to 48-hour and the 72- to 96-hour sampling intervals.

B. A quality assurance statement was signed and dated October 12, 1937.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The major deficiency with this study is that total recovery of radioactivity accounted for only about 50 percent of the dose. Consequently, the data are of qualitative value since it cannot be determined whether the balance was lost or is sequestered in various tissues. An attempt was made to analyze more tissues and the remaining carcass; however, recovery of a few μCi is difficult in larger animals. Nonetheless, the data indicate that at least 20 percent (and possibly up to 75 percent) of the applied dose is absorbed within 10 hours after application of 2.2- to 3.0-mg/kg doses. This study provides supplementary data.

Data Evaluation Report

Compound Endosulfan

Citation
A dermal absorption study in rats with ^{14}C-endosulfan with extended test duration, E.M. Craine, WIL Research Labs., WIL 39029, 11/17/88, MRID 410485-04

Reviewed by Robert P. Zendzian Ph.D. 1/12/96
Senior Pharmacologist

Core Clasification Acceptable

Conclusions

Female rats were dosed dermally at 1.9, 21.9 and 231.4 mg/cm^2, waashed at 10 hours and followed to 168 hours. 44.8, 46.4 and 20.3 % of the respective doses was absorbed.

Materials

^{14}C ring labeled endosulfan, from Hoerst Aktienesekkschaft
Preparation 16014, Code HOE 002671 OI ZE98 0002, 27.2 uCi/mg, radiopurity 94.6%, [redacted]
Preparation 16014 I, Code HOE 002671 OI ZE98 0003, 5.47 uCi/mg, radiopurity 94.6%, [redacted]

Blank formulation, for Thiodan 3CE, E 4847:27, code 519

Female Crl:CD®(SD)BR for Charles River, 7-10 weeks of age

Experimental Design

Rats were exposed for 10 hours, the applicaton site washed and terminated as follows:

Group Number	Number Rats	Dose mg/kg	Dose mg/rat	Wash (hrs)	No. Rats at Termination (hr) 24	48	72	168
I	16	0.1	0.021	10	4	4	4	4
II	16	1.0	0.237	10	4	4	4	4
III	16	10.0	2.500	10	4	4	4	4

Dosing

Radiolabeled endosulfan was suspended in the formulation blank. Suspensions were analyzed and dosing volumes based on the analytical concentration.

On the day before dosing the hair was clipped from an area on the rats back. About 30 minutes before dosing the clipped area was washed with acetone. "a rubber ring (inside diameter = 3.7 cm, area inside the ring = 10.8 cm^2, width = 1 cm) was cemented to the shaved skin with cyananoacrylate glue forming an application site. The dose of test material, was applied evenly to the application site with a positive displacement glass pipet (Micro-Petter). The pipet was washed with

ethanol. The amount of ^{14}C in the wash was determined to quantitate the applied dose. A circle of Whatman #1 filter paper (5.5 cm in diameter) was cemented to the rubber ring covering the application area using "liquid nails" (SCM Macco Adhesive)." Rats were placed individually in Nalgene metabolism units for collection of urine and feces.

Ten hours after dosing the filter paper was removed and the application site washed with 1% Liquid Ivory and rinsed with deionized water. Rats were returned to the metabolism cages. At the end of the absorption period individual rats were euthanized with nitrogen and a 4 ml blood sample removed from the vena cava. The application site skin with rubber ring attached, the liver, both kidneys, about 1.0 gm subcutaneous fat, muscle, the entire brain, a section of untreated skin and the residual carcass were collected for analysis. Residual urine in the bladder was added to the urine collection. Total urine and feces were collected for analysis.

The following samples were analyzed for each rat;

skin wash	brain	liver	carcass
filter paper	fat	kidney	
rubber ring	muscle	Urine	
Application site skin	blood	feces	

Results

Table 2 from the report presents the mean concentrations of endosulfan equilivants in the tissue samples. Table 3 from the report presents the mean disposition of endosulfan in the treatment groups..

Discussion

This study was performed with two variations from the standard experimental design which can be expected effect the data generated. The performing laboratory is aware of the standard design and did not present reasons for the variations. Female rats, rather than males were used. We have some experimental data to indicate that there are significant sex related differences in skin permeability. The standard design requires cleaning the application site with acetone 24 hours before dosing. This removes surface fats and oils and extracts some lipoid material from the skin. In 24 hours the extracted material is replaced by the skin. In this study the acetone wash was performed 30 minutes before dosing. Thus, the application site is abnormally deficient in lipoid material to an extent that can be expected to effect its permeability. At this time we do not have the data necessary to prediction the direction and magnitude of the loss of lipoid material on the dermal penetration of the test material.

39029.15
PROJECT WIL-39029

Table 7

Summary: The average concentrations of endosulfan equivalents in whole blood and tissues of sub groups of four animals sacrificed after specified periods of time. The symbol "less than" (<) indicates concentration below a reliable measurable level.

Dose group	Time of sacrifice (hrs)	Average concentrations of endosulfan equivalents in tissues collected and analyzed					
		Liver (ug/g)	Kidney (ug/g)	Brain (ug/g)	Fat (ug/g)	Muscle (ug/g)	Blood (ug/g)
I	24.0	0.08	0.70	0.01	0.04	0.01	<0.01
	48.0	0.09	1.08	0.01	0.04	<0.01	<0.01
	72.0	0.06	0.97	<0.01	0.02	<0.01	<0.01
	168.0	0.02	0.64	<0.01	<0.02	<0.01	<0.01
II	24.0	0.82	5.82	0.15	0.48	0.05	0.03
	48.0	1.21	11.56	0.13	0.67	0.06	0.04
	72.0	0.72	2.50	0.06	0.26	0.03	0.03
	168.0	0.47	2.40	0.03	0.06	0.01	0.02
III	24.0	2.35	4.29	0.42	1.24	0.12	0.08
	48.0	5.92	14.03	0.79	2.86	0.22	0.18
	72.0	3.76	11.01	0.40	1.95	0.14	0.12
	168.0	2.36	11.80	0.14	0.30	0.02	0.10

W 029
Hoechst AG

TABLE 3

Summary: The average disposition of applied ^{14}C-endosulfan for the sub-groups of four rats dosed at the same level of endosulfan and sacrificed at the same time interval after initial exposure to the endosulfan. Column heading numbers indicate the time of sacrifice of the sub-group following initial exposure.

	GROUP I				GROUP II				GROUP III			
	24 hr.	48 hr.	72 hr.	168 hr.	24 hr.	48 hr.	72 hr.	168 hr.	24 hr.	48 hr.	72 hr.	168 hr.
Dose applied (µg)	20.9	20.7	21.0	21.0	236	237	238	237	2512	2482	2510	2505
Dose applied (mg/kg)	0.08	0.09	0.09	0.09	0.99	0.98	0.99	0.98	11.33	10.93	10.97	10.67
Dose removed by washing (%)	30.7	35.0	27.7	28.0	40.0	51.1	42.5	46.8	59.7	70.2	65.0	68.6
Protective Device (% of dose)	12.3	11.2	10.5	11.9	19.2	13.1	13.7	12.2	2.1	8.5	6.7	3.3
Amount in application site skin (% of dose)	41.4	23.8	7.8	1.7	39.0	17.3	11.8	1.5	33.0	20.2	13.4	1.0
Amount not penetrated (% of dose)	84.5	70.0	45.2	41.6	99.1	81.6	68.0	56.2	94.8	98.9	85.1	72.8
Amount in the urine (% of dose)	3.5	7.9	10.4	13.7	2.7	7.2	7.2	13.1	0.73	2.40	2.82	5.82
Amount in feces (% of dose)	5.6	14.8	21.9	28.6	3.2	13.6	15.2	31.1	0.6	3.2	5.6	13.2
Total excreted (% of dose)	9.0	22.8	32.4	42.3	5.8	20.8	22.4	44.2	1.4	5.6	8.4	19.0
Amount in the animal (% of dose)	13.0	12.6	6.6	2.5	10.2	15.3	7.4	2.3	2.5	5.5	3.6	1.4
Total penetrated (µg)	4.63	7.32	8.16	9.40	37.94	85.70	70.94	110.20	95.63	275.63	302.28	508.01
Flux of penetration (µg/sq cm/hour)	0.018	0.014	0.010	0.005	0.148	0.165	0.091	0.061	0.369	0.532	0.389	0.280
Total penetrated (% of dose)	22.1	35.3	39.0	44.8	16.1	36.2	28.7	46.3	3.8	11.1	12.0	20.3
Total excreted (% of penetrated)	41	64	83	94	36	57	78	95	37	50	70	94
Total recovered (% of dose)	106	106	84	86	115	118	98	103	99	110	97	93

007937

EPA No.: 68D80056
DYNAMAC No.: 210-F
TASK No.: 2-10F
January 10, 1990

DATA EVALUATION RECORD

ENDOSULFAN

Chronic Toxicity/Carcinogenicity Feeding Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 1/10/90

007937

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ENDOSULFAN

Chronic Toxicity/Carcinogenicity
Feeding Study in Rats

REVIEWED BY:

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DATA EVALUATION RECORD

GUIDELINE §83-5

STUDY TYPE: Chronic toxicity/carcinogenicity feeding study in rats.

MRID NUMBER: 410995-02.

TEST MATERIAL: Endosulfan.

SYNONYM(S): Thiodan; benzoepin; endocide; 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide.

STUDY NUMBER(S): HST 289/881067.

SPONSOR: Hoechst Celanese Corporation, North Somerville, NJ.

TESTING FACILITY: Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, PE18, 6ES, England.

TITLE OF REPORT: Combined Chronic Toxicity/Carcinogenicity Study--104-Week Feeding in Rats.

AUTHOR(S): Ruckman, S., Waterson, L., Crook, D., Gopinath, C., Majeed, S., Anderson, A., and Chanter, D.

REPORT ISSUED: April 1, 1989.

CONCLUSIONS:

Under the conditions of the study, endosulfan was not oncogenic when administered to Sprague-Dawley rats for 2 years at dietary levels of 0, 3.0, 7.5, 15.0, or 75.0 ppm.

No effects of dosing on clinical signs, mortality, food and water consumption, ophthalmological examinations and urinalyses were observed. Mean body weight gains were significantly decreased ($p < 0.01$) when compared with controls in males and females receiving 75.0 ppm. No toxicologically important changes in hematology and clinical chemistry parameters were observed. An increased incidence of bilaterally enlarged kidneys in females receiving 75.0 ppm was seen on gross examination. Aneurysms of blood vessels and enlarged lumbar lymph nodes were also observed with increased incidences in satellite group male rats receiving 75.0 ppm. Other grossly observed pathological findings occurred sporadically and were considered to be incidental. Organ weights were not affected by dosing. Histopathologically, an increased incidence of marked progressive glomerulonephrosis in the kidneys and an increased incidence of aneurysms of the blood vessels were seen in male rats receiving 75.0 ppm endosulfan. Neoplastic lesions had a similar incidence in control and dosed groups and were considered incidental.

The LOEL is 75.0 ppm (3.45 mg/kg/day) based on reduced body weight gain in males and females, and/or an increased incidence of marked progressive glomerulonephrosis and of blood vessel aneurysms in males. The NOEL was 15.0 ppm (0.65 mg/kg/day) when endosulfan was fed to rats for 2 years.

Classification: CORE minimum.

A. MATERIALS:

1. Test Compound: Endosulfan (technical); description: buff colored flakes; batch No.: 381(A-D); purity: 97.1%.

2. Test Animals: Species: rat; strain: CD(SD)BR; age: 4 weeks; weight: males--23 g and females--27 g; source: Charles River Breeding Laboratories, Portage, MI.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimatized for 5 days and their health was observed. Males and females free of abnormal signs were assigned to one of the following four

test groups or a control group using a computer randomization method with consideration of approximate homogeneous group weights:

Test group	Dose in diet (ppm)	Main study (24 months) Males	Main study (24 months) Females	Satellite Groups[a] Males	Satellite Groups[a] Females
1 Control	0	50	50	20	20
2 Low (LDT)	3.0	50	50	20	20
3 Mid-1 (MDT)	7.5	50	50	20	20
4 Mid-2 (MDT)	15.0	50	50	20	20
5 High (HDT)	75.0	50	50	20	20

[a]These animals were intended for toxicity evaluation and were sampled at intervals for hematology and clinical chemistry; survivors were sacrificed after 104 weeks. An additional group of 10 rats/sex were used for pretest hematology and health check.

The above dose levels were selected from the results of a 90-day study (HRC Report No. HST 204/83763) and a multigeneration study (HRC Report No. HST 230/84176); neither the results nor the dose levels were reported.

Animals were housed five to a cage, and after proper identification and labeling, cages were placed in a temperature- and humidity-controlled room with a 12-hour light/dark cycle.

2. Diet Preparation: A premix of endosulfan in the diet was prepared weekly by dissolving endosulfan in acetone, then mixing in corn oil and adding to Labsure Laboratory Animal Diet No. 2. The concentrations were adjusted by direct dilution of the premix with untreated diet and mixing in a double cone blender for a minimum of 7 minutes. The concentration of test article in the diet was determined during weeks 1, 12, 25, 38, 51, 65, 77, 90, and 104 of the study. The mixtures containing the test article were analyzed for homogeneity and stability.

Results: Table 1 summarizes mean concentrations of endosulfan in test diets analyzed in duplicate at eight intervals during the study. All diets were within 10% of the nominal concentration. Diets were reported to be homogeneous and endosulfan was demonstrated to be stable for up to 18 days under storage conditions. The relative standard deviations for homogeneity were 4.1, 1.6, and 1.6% for diets at 3, 10, and 75 ppm (These do not correspond to levels used in the study).

TABLE 1. Mean Analyzed Concentrations of Endosulfan in Diets of Rats[a]

Nominal Level (ppm)	Mean Analyzed Concentration (ppm ± S.D.)
3.0	3.00 ± 0.079
7.5	7.09 ± 0.231
15.0	14.35 ± 0.410
75.0	73.01 ± 2.02

[a]Data were extracted from Study No. HST/289, Addendum 3.

3. Food and Water Consumption: Animals received food (Labsure Laboratory Animal Diet No. 2) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the data: food consumption, water consumption, body weight, organ weight, and clinical pathology data were analyzed by frequency analysis if data consisted predominantly of one particular value using Fisher's and Mantel's tests; otherwise, Bartlett's test was applied to detect heterogeneity of variance between treated groups. If no significant heterogeneity was detected, a one-way analysis of variance (ANOVA) was carried out. If heterogeneous variances could not be removed by log transformation, the data were further examined using the Kruskal-Wallis analysis of rank. ANOVA was followed by Student's t-test and Williams' test for a dose-related response. The Kruskal-Wallis analyses were followed by a non-parametric equivalent of the t-test or by the Shirley's test.

   Food consumption data were analyzed on a cumulative cage basis and body weight data were analyzed using weight gains.

   Analysis of covariance was used in place of analysis of variance for organ weight data where appropriate, using final body weight as the covariate. Mortality data were analyzed using the log ranks method.

5. Quality Assurance: A quality assurance statement was signed and dated March 29, 1989.

C. METHODS AND RESULTS:

1. Observations: All animals were observed daily every weekday for the first 4 weeks of the study; thereafter, they were examined once weekly. Each rat received a detailed clinical examination including signs of ill health, toxicity, behavioral effects, mortality, and moribundity. In addition, all rats were palpated for tissue masses and a weekly record of progression or regression of these masses was maintained.

   Results: No treatment-related signs of toxicity or clinical symptoms were reported. The most common findings were brown fur staining, hair loss, hunched posture, pale extremities, prominent vertebrae, and piloerection. All these findings were considered to be incidental, and were observed in dosed as well as control groups. A summary tabulation of clinical observations was not provided, but

findings were recorded on the individual animal pathology sheets (See Reviewer's Discussion, Section E.)

Table 2 presents mortality incidence and percent survival in the main study. No indication of a treatment-related effect on mortality was observed. No dose-related increases in mortality were seen in the animals of main study. At 78 weeks, survival ranged between 74 and 90% in all groups of males and between 78 and 90% in all groups of females. At termination, survival in male groups ranged from 38 to 42% and in female groups from 28 to 42%.

In the satellite groups, total mortality was reported to be 7, 11, 14, 16, and 14 in males and 16, 12, 10, 14, and 10 in females receiving 0, 3.0, 7.5, 15.0, or 75.0 ppm, respectively. However, examination of the individual histopathology data indicated that the terminal sacrifice for some satellite rats was after week 104 and it was not completed until week 107. Several deaths occurred in the period between week 103-107 (See Reviewers Discussion).

2. Body Weight: Body weights were recorded prior to study initiation, at study initiation, and every week thereafter through study week 104.

Results: Table 3 presents mean body weight changes between selected weekly intervals. Mean weight gains tended to be decreased in both males and females receiving 15.0 and 75.0 ppm. Weight gains were significantly depressed ($p < 0.05$) during weeks 6-18 in males receiving 15.0 and 75.0 ppm when compared with controls; decreases did not achieve a level of significance in males or females receiving 15.0 ppm at other intervals of analysis.

Between weeks 0 and 64, body weight gains were 9% and 13% lower in males and females of 75.0-ppm groups, respectively, than those of the controls. Overall weight gains (weeks 0-104) were 17% lower than the controls in both males and females receiving 75.0 ppm endosulfan and 9% lower in rats receiving 15.0 ppm. The gains were significantly lower ($p < 0.01$) in both sexes at the 75.0-ppm dose. No weight gain effects were seen in males and females receiving 3.0 or 7.5 ppm when compared with controls.

TABLE 2. Cumulative Mortality Incidences and Percent Survival in Rats Fed Endosulfan for 2 Years[a,b]

Dietary Level (ppm)	Cumulative Mortality and (Percent Survival) at Weeks:					
	Males			Females		
	52	78	103	52	78	104
0	2 (96)	9 (82)	29 (42)	0 (100)	7 (86)	36 (28)
3.0	2 (96)	11 (78)	31 (38)	1 (98)	9 (82)	30 (40)
7.5	1 (98)	5 (90)	30 (40)	3 (94)	12 (76)	32 (36)
15.0	0(100)	10 (80)	30 (40)	0 (100)	5 (90)	29 (42)
75.0	2 (96)	11 (74)	31 (38)	3 (94)	11 (78)	32 (36)

[a]Data were extracted from study No. HST 289/881067, Addendum 4.

[b]Based on 50 rats/sex/group in the main study.

TABLE 3. Mean Body Weight Changes (± S.D.) Between Selected Weekly Intervals for Rats Fed Endosulfan for 2 Years[a]

Dietary Level (ppm)	Selected Weekly Intervals During Treatment Period					
	0-6	6-18	18-64	64-104	0-64	0-104
			Males			
0	230 ± 30.0	160 ± 28.0	200 ± 55.9	-11 ± 103.4	590 ± 91.5	580 ± 124.0
3.0	233 ± 33.3	157 ± 31.8	203 ± 72.8	13 ± 99.7	594 ± 109.3	570 ± 125.4
7.5	234 ± 36.7	159 ± 38.7	194 ± 76.9	-9 ± 113.0	586 ± 127.6	531 ± 131.3
15.0	226 ± 30.6	145 ± 32.2**	193 ± 74.5	9 ± 102.4	564 ± 107.8	525 ± 115.4
75.0	229 ± 30.7	144 ± 31.6**	163 ± 76.5*	-43 ± 107.3	536 ± 105.8**	479 ± 94.1**
			Females			
0	113 ± 20.7	69 ± 17.2	153 ± 67.4	94 ± 117.1	337 ± 88.1	398 ± 104.7
3.0	110 ± 18.3	66 ± 19.0	146 ± 57.7	69 ± 101.9	322 ± 79.3	350 ± 107.3
7.5	113 ± 17.6	69 ± 19.2	153 ± 59.6	80 ± 67.4	334 ± 82.6	414 ± 84.7
15.0	108 ± 16.6	68 ± 18.2	143 ± 57.3	82 ± 76.9	318 ± 77.4	363 ± 91.9
75.0	104 ± 15.5**	65 ± 15.0	125 ± 61.7**	56 ± 64.6	294 ± 76.3**	328 ± 100.0*

Data were extracted from study No. HST 289/881067, pp. 40-44.

*Significantly different from control values ($p < 0.05$).

**Significantly different from control values ($p < 0.01$).

3. Food and Water Consumption, Food Efficiency, and Compound Intake: Food consumption was recorded weekly on a cage basis. Water consumption was measured by weight over daily periods during weeks 11, 26, and 50 for all satellite group animals. Food efficiency was calculated from the body weight and food consumption data. The mean compound intake was calculated daily for each week.

   Results: Table 4 summarizes mean food consumption data between selected weekly intervals. No apparent treatment-related effects were noted in food and water consumption among males or females at all dietary levels. Although males receiving 15.0 or 75.0 ppm showed a tendency for slightly lower mean food consumption (3 to 4%) from weeks 1 to 64, significantly reduced ($p < 0.05$) mean values of food consumption when compared with controls at these dose levels were noticed between weeks 65 and 104; the reductions were sporadic, were only marginal (5-6%), and were not considered to be of importance. No treatment-related effects on food conversion ratios were found, although data for females receiving 75.0 ppm indicated marginally decreased efficiency compared with controls for the first 26 weeks.

   The calculated mean achieved intakes for the entire study were 0.1, 0.3, 0.6, or 2.9 mg/kg body weight/day for males receiving dietary levels of 3.0, 7.5, 15.0, or 75.0 ppm and 0.1, 0.4, 0.7, or 3.8 mg/kg body weight/day for females receiving the same dietary levels of endosulfan.

4. Ophthalmological Examinations: Ophthalmological examinations were conducted for animals of control and high-dose groups prior to study initiation, during week 50, and at study termination (week 104).

   Results: No significant differences in ophthalmological findings were observed between control and high-dose groups.

5. Hematology and Clinical Chemistry: Prior to study initiation, a routine hematological evaluation was performed on 10 rats/sex allocated to the health check group. Blood was collected from the 10 fasted rats/sex/group via orbital sinus under light ether anesthesia during weeks 13, 26, 52, 78 and prior to study termination (week 103). Samples were obtained from satellite group rats through week 78. Terminal sampling included rats of the main group where necessary to maintain the sample size of 10 rats/sex/group. The CHECKED (X) parameters were examined:

TABLE 4. Mean Food Consumption Data (g/rat ± S.D.) Between Selected Weekly Intervals for Rats Fed Endosulfan for 2 Years[a]

Dietary Level (ppm)	Selected Weekly Intervals During Treatment Period					
	1-6	7-18	19-64	1-64	65-104	1-104
			Males			
0	1136 ± 60	2210 ± 150	8084 ± 608	11430 ± 810	7082 ± 514	18511 ± 1270
3.0	1134 ± 36	2192 ± 84	8030 ± 342	11356 ± 447	7053 ± 391	18396 ± 744
7.5	1136 ± 48	2208 ± 117	8022 ± 416	11366 ± 571	6894 ± 310	18259 ± 793
15.0	1114 ± 44	2141 ± 95	7847 ± 438	11101 ± 565	6644 ± 344*	17752 ± 753
75.0	1118 ± 45	2137 ± 111	7788 ± 411	11042 ± 539	6743 ± 414*	17790 ± 870
			Females			
0	852 ± 25	1580 ± 49	6020 ± 226	8452 ± 282	5799 ± 384	14233 ± 623
3.0	851 ± 44	1583 ± 84	6000 ± 321	8434 ± 428	5560 ± 338	14005 ± 727
7.5	868 ± 30	1627 ± 86	6180 ± 373	8675 ± 477	5976 ± 500	14640 ± 893
15.0	854 ± 61	1602 ± 120	6088 ± 508	8545 ± 681	5852 ± 632	14397 ± 1283
75.0	820 ± 42	1579 ± 99	5945 ± 407	8343 ± 536	5654 ± 358	13997 ± 853

Data were extracted from study No. HST 289/881067, pp. 45-49.
*Significantly different from control values ($p < 0.05$).

a. Hematology:

X	Hematocrit (HCT)†	X	Leukocyte differential count
X	Hemoglobin (HGB)†		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)†	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)†	X	Mean corpuscular volume (MCV)
X	Platelet count†	X	Coagulation:thromboplastin time (PT)
	Reticulocyte count (RETIC)		
X	Red cell morphology		

Results: Table 5 presents selected mean hematology data at 13, 52 and 103 weeks. The study authors reported that no treatment-related changes were noted between control and treated groups in hematology parameters at 13, 26, 52, 78 and 103 weeks of study.

At week 13, RBC counts were increased ($p < 0.01$) and there was a corresponding decrease of MCV in all dosed groups of females compared to controls. However, there were no changes at any other interval of sampling in females and no effects in males. All values were also within the normal range, so the effect was not considered to be of toxicologic importance.

Significant decreases ($p \leq 0.05$) in total leukocyte counts (at week 26) and lymphocyte counts (at weeks 26 and 52) were noted in males receiving 75.0 ppm when compared to controls. These decreases were marginal, were not consistent over intervals or dose-related, and were within the normal range of background data provided by the study laboratory.

All other changes that reached a level of significance were considered to be incidental and were within the normal range of biological variation.

†Recommended by Subdivision F (October 1982) Guidelines.

TABLE 5. Selected Mean Hematology Data at 13, 52, and 103 Weeks Samplings for Rats Fed Endosulfan for 2 Years[a,b,c]

Dietary Level (ppm)	Selected Mean Hematology Data at Sampling Periods: HCT (%)			HGB (g/dL)			RBC (10^6/mm^3)			MCV (fl)		
	13-week	52-week	103-week	13-week	52-week	103-week	13-week	52-week	103-week	13-week	52-week	103-week
Males												
0	53 ± 2.5	49 ± 2.0	45 ± 7.7	15.7 ± 0.74	14.9 ± 0.09	13.0 ± 2.35	9.0 ± 0.53	7.8 ± 0.38	6.3 ± 1.07	60 ± 2.1	62 ± 2.2	72 ± 1.6
3.0	53 ± 1.2	49 ± 2.6	49 ± 3.7	15.7 ± 0.45	15.2 ± 0.81	13.9 ± 0.97	8.4 ± 0.30	7.6 ± 0.46	6.8 ± 0.60	63 ± 2.7	65 ± 2.9	72 ± 2.3
7.5	53 ± 1.6	49 ± 2.2	45 ± 4.3	15.5 ± 0.33	15.3 ± 1.38	13.3 ± 1.26	8.5 ± 0.37	7.6 ± 0.56	6.4 ± 0.70	62 ± 2.4	65 ± 3.0	70 ± 2.6
15.0	53 ± 1.3	49 ± 2.1	45 ± 5.8	15.9 ± 0.28	15.1 ± 0.66	13.0 ± 1.80	8.9 ± 0.45	7.7 ± 0.31	6.4 ± 0.78	60 ± 2.2	64 ± 3.3	71 ± 1.3
75.0	52 ± 1.4	48 ± 1.5	45 ± 6.9	15.5 ± 0.26	14.9 ± 0.47	12.7 ± 2.03	8.7 ± 0.39	7.8 ± 0.30	6.6 ± 0.82	60 ± 2.1	62 ± 2.3	68 ± 4.0*
Females												
0	52 ± 1.9	47 ± 2.4	47 ± 5.1	15.6 ± 0.57	14.4 ± 0.77	13.8 ± 1.28	7.5 ± 0.43	6.9 ± 0.45	6.6 ± 0.85	69 ± 5.0	6.9 ± 1.9	71 ± 2.1
3.0	52 ± 1.6	47 ± 1.6	44 ± 4.4	15.5 ± 0.25	14.7 ± 0.46	12.9 ± 1.56	8.3 ± 0.35**	6.9 ± 0.21	6.3 ± 0.60	62 ± 1.6**	6.9 ± 2.1	71 ± 1.2
7.5	51 ± 1.6	48 ± 1.9	45 ± 3.3	15.3 ± 0.41	14.7 ± 0.63	13.6 ± 0.78	8.4 ± 0.48**	7.0 ± 0.36	6.4 ± 0.50	61 ± 2.1**	6.9 ± 1.6	70 ± 1.1
15.0	52 ± 1.4	47 ± 2.2	45 ± 3.6	15.9 ± 0.55	14.3 ± 0.65	13.6 ± 1.13	8.6 ± 0.46**	6.8 ± 0.31	6.5 ± 0.54	61 ± 2.2**	6.9 ± 2.2	69 ± 2.7
75.0	50 ± 1.3*	47 ± 1.9	44 ± 7.1	15.6 ± 0.40	14.3 ± 0.69	13.0 ± 1.91	8.2 ± 0.37**	6.8 ± 0.30	6.1 ± 0.97	62 ± 1.7**	68 ± 1.4	71 ± 1.3

[a]Data were extracted from study No. HST 289/881067, pp. 58-62.

[b]Abbreviations are as follows:

HCT: Hematocrit;
HGB: Hemoglobin;
RBC: Erythrocyte count;
MCV: Mean corpuscular volume.

[c]Standard deviations were calculated by the reviewers.

*Significantly different from control values ($p < 0.05$).

**Significantly different from control values ($p < 0.01$).

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†		Albumin/globulin ratio
	Magnesium†	X	Blood creatinine†
X	Phosphorus†	X	Blood urea nitrogen†
X	Potassium†		Cholesterol†
X	Sodium†	X	Globulins
		X	Glucose†
	Enzymes		Total bilirubin†
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein†
	Creatinine phosphokinase†		Triglycerides
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		
	Gamma glutamyltransferase (GGT)		

Results: The study authors reported that there were no treatment-related changes in the clinical chemistry parameters throughout the study.

Slight alteration in the protein parameters (total protein, albumin, and globulin) and serum electrolyte levels (calcium, sodium, potassium, phosphorus, and chloride) reached a level of significance in the treated groups when compared to controls; these effects were not consistent over intervals of sampling, nor were they dose-related. These differences were generally within the normal range of background data provided by the study laboratory.

6. Urinalysis: Urinalyses were performed during weeks 12, 25, 51, 77, and 102 by collecting overnight urine samples. Drinking water was withheld during urine collection. The CHECKED (X) parameters were examined:

X	Appearance†	X	Glucose†
X	Volume†	X	Ketones†
X	Specific gravity†	X	Bilirubin†
X	pH	X	Blood†
X	Sediment (microscopic)†	X	Nitrate
X	Protein†	X	Urobilinogen

†Recommended by Subdivision F (October 1982) Guidelines.

Results: Table 6 summarizes results of selected urinary parameters at the 102-week interval of sampling. Significantly increased urinary protein concentrations were noted in males receiving 15.0 ($p < 0.05$) and 75.0 ($p < 0.01$) ppm at week 102 when compared with controls. Increases were observed only in a few of the males and only at the terminal sampling, and they were not observed in females. The increased values were within the normal biological variation and were not considered to be of toxicologic importance.

7. Sacrifice and Pathology: All animals that died or were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected and fixed. In addition, the (XX) organs were weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta†	XX	Brain
X	Salivary glands†	XX	Heart†	X	Peripheral nerve (sciatic nerve)†
X	Esophagus†	X	Bone marrow†		
X	Stomach†	X	Lymph nodes†	X	Spinal cord (3 levels)
X	Duodenum†	X	Spleen	XX	Pituitary†
X	Jejunum†	X	Thymus	X	Eyes (optic nerve)†
X	Ileum†				
X	Cecum†				
X	Colon†				
X	Rectum		Urogenital		Glandular
XX	Liver†	XX	Kidneys†	XX	Adrenals†
X	Gallbladder†	X	Urinary bladder†		Lacrimal gland
X	Pancreas†	XX	Testes†	X	Mammary gland†
		X	Epididymides	XX	Thyroids†
		X	Prostate	X	Parathyroids†
		X	Seminal vesicle		Harderian glands
	Respiratory	XX	Ovaries		
X	Trachea†	X	Uterus		
X	Lung†				
					Other
				X	Bone (sternum and femur)†
				X	Skeletal muscle†
				X	Skin
				X	All gross lesions and masses

†Recommended by Subdivision F (October 1982) Guidelines.

TABLE 6. Results of Selected Urinary Parameters in Rats Fed Endosulfan, Sampled Prior to Study Termination During Week 102[a,b,c]

Dietary Level (ppm)	Results of Selected Urinary Parameters Sampled During Week 102: Males Volume (mL)	pH	Specific Gravity (x1000)	Protein (mg/dL)	Females Volume (mL)	pH	Specific Gravity (x1000)	Protein (mg/dL)
0	11.9±4.8	6.3±0.58	1034±8.7	375±178	7.4±4.3	6.2±0.28	1033±6.6	197±144
3.5	8.6±3.7	6.1±0.25	1041±5.3	400±149	8.2±5.2	6.1±0.39	1031±4.8	322±346
7.5	7.2±2.9	6.1±0.21	1042±7.7	533±206	12.0±3.5	6.1±0.22	1031±5.5	351±249
15.0	12.3±5.8	5.9±0.35*	1038±7.3	600±221*	10.6±4.4	6.2±0.28	1029±5.7	207±147
75.0	9.6±7.3	6.0±0.31*	1040±6.2	620±162**	11.8±4.3*	6.0±0.21	1028±3.5*	348±289

[a]Data were extracted from study No. HST 289/881067, p. 68 and Table 8.

[b]Samples were obtained from 10 rats/sex from each surviving group.

[c]Standard deviations were calculated by the reviewers.

*Significantly different from control values ($p < 0.05$).

**Significantly different from control values ($p < 0.01$).

Lung, liver, and kidneys were examined histologically in all groups at termination. All checked tissues were examined for animals in the control and 75-ppm groups (both satellite and main groups). In the groups receiving 3, 7.5, and 15 ppm, a complete complement of tissues was only examined for rats that died or were sacrificed moribund.

Results:

a. Organ Weights: The study authors reported that there were no treatment-related changes in organ weights at termination. Mean testicular weights were significantly decreased ($p < 0.01$) in males receiving 15.0 or 75.0 ppm when compared with controls. These decreases were within the normal background range provided by the study laboratory and were not considered to be of toxicological importance.

   There were no other apparent differences in other organ weights, comparing control and treated rats of either sex. No notable effects were observed in organ weight data of animals that died prior to the terminal sacrifice.

b. Gross Pathology: Tables 7 and 8 summarize selected frequent gross pathological findings in rats of the satellite groups and main study, respectively.

   The incidence of bilaterally enlarged kidneys was increased in females of both satellite and main groups receiving 75.0 ppm when compared with controls. Other findings in the kidneys (paleness, irregular or uniform cortical scarring, and cysts) occurred at similar frequencies in control and dosed groups.

   The incidence of aneurysms in blood vessels was increased in satellite males receiving 75.0 ppm when compared with controls; in the main group, the increase at the same dose was not marked.

   A variety of other gross pathology findings occurred sporadically in both control and dosed groups and were not considered related to dosing.

c. Microscopic Pathology:

   1) Nonneoplastic: Table 9 summarizes frequent nonneoplastic lesions combining data for rats in the satellite and main groups.

TABLE 7. Selected Gross Pathological Findings in Satellite Group Rats Fed Endosulfan for 2 Years[a,b]

Gross Pathological Findings	Dietary Level (ppm)									
	Males					Females				
	0	3.0	7.5	15.0	75.0	0	3.0	7.5	15.0	75.0
No. animals examined	20	20	20	20	20	20	20	20	20	20
Blood Vessels										
Aneurysm(s)	1	2	2	3	6	0	0	1	1	0
Kidneys										
Paleness	2	7	4	3	4	2	2	1	2	4
Enlarged, bilateral	9	8	12	8	12	2	6	5	4	8
Cortical scarring, uniform	7	11	13	9	13	3	6	6	4	6
Cortical scarring, irregular	0	1	0	0	1	1	1	2	1	0
Cyst(s)	3	2	2	3	3	0	0	1	1	0
Lumbar Lymph Nodes										
Enlarged (cystic)	2	2	3	2	5	1	0	0	1	1
Lungs										
Pale focus	5	2	4	2	1	4	2	0	3	5
Petechial	3	2	1	1	1	4	1	3	2	2
Congested	3	2	6	3	3	2	6	1	2	4
Liver										
Enlarged	2	4	5	1	2	1	2	6	1	5
Dark area(s)	14	16	15	14	12	13	16	18	16	16
Swollen	10	12	9	8	7	14	17	14	12	14
Testes										
Small	2	4	6	3	2					
Flaccid	1	3	2	2	4					
Ovaries										
Cyst(s)						4	2	1	0	4
Subcutaneous Mass										
Mass(es)	4	5	9	5	5	12	18	17	15	18

[a]Data were extracted from study No. HST 289/881067, pp. 83-91 and Table 9b.

[b]20 rats/sex/group were examined; this total includes rats found dead or sacrificed in extremis and those sacrificed after 104 weeks of treatment (Satellite term).

TABLE 8. Selected Gross Pathological Findings in Rats of Main Study Fed Endosulfan for 2 Years[a,b]

Gross Pathological Findings	Dietary Level (ppm)									
	Males					Females				
	0	3.0	7.5	15.0	75.0	0	3.0	7.5	15.0	75.0
No. animals examined	50	50	50	50	50	50	50	50	50	50
Blood Vessels										
Aneurysm(s)	9	3	10	5	12	0	0	1	2	3
Kidneys										
Paleness	12	10	13	12	10	6	4	7	6	9
Enlarged, bilateral	29	24	27	26	27	8	12	14	13	18
Cortical scarring, uniform	27	24	34	32	31	12	6	12	9	14
Cortical scarring, irregular	1	5	3	2	5	5	5	5	3	3
Cyst(s)	4	8	7	7	9	0	1	0	3	2
Lumbar Lymph Nodes										
Enlarged (cystic)	12	8	5	5	14	0	2	1	1	2
Lungs										
Pale focus	6	6	9	9	8	7	7	5	11	9
Petechial	2	6	4	6	7	6	10	7	5	3
Congested	6	7	4	3	4	5	5	5	6	9
Liver										
Mass(es)	3	1	1	3	3	0	1	1	0	1
Enlarged	6	4	8	4	6	7	8	12	6	5
Dark area(s)	39	37	36	39	30	35	38	39	42	34
Swollen	35	22	22	29	22	37	35	36	37	27
Testes										
Small	5	4	9	11	7					
Flaccid	4	6	9	8	6					
Ovaries										
Cyst(s)	-					5	9	6	11	10
Subcutaneous Mass										
Mass(es)	11	11	15	9	6	40	40	42	35	35

[a]Data were extracted from study No. HST 289/881067, pp. 73 and 83 and Table 9a.

[b]Fifty rats/sex/group were examined; this total includes rats found dead or sacrificed in extremis and those sacrificed at termination (104 weeks).

TABLE 9. Frequent Nonneoplastic Lesions in Rats Fed Endosulfan for 2 Years[a,b]

Organ/Finding	Dietary level (ppm)									
	Males					Females				
	0	3.0	7.5	15.0	75.0	0	3.0	7.5	15.0	75.0
Blood Vessels[c]										
Aneurysm(s)	10	6	14	10	19	1	2	5	4	3
Kidneys	(70)[d]	(70)	(70)	(70)	(70)	(70)	(70)	(70)	(70)	(70)
Progressive glomerulonephrosis	55	52	64	61	44	29	37	42	39	37
Minimal/moderate:	35	34	42	37	28	28	31	36	34	29
Marked:	20	18	22	24	30	1	6	6	5	8
Heart	(70)	(50)	(51)	(55)	(70)	(70)	(48)	(56)	(52)	(70)
Myocardial scarring	48	37	31	31	40	45	21	23	22	23
Spleen	(70)	(45)	(48)	(47)	(70)	(70)	(44)	(45)	(46)	(70)
Hemosiderosis	40	25	18	22	36	33	27	28	28	47
Ovaries						(70)	59	50	(59)	(70)
Tubular hyperplasia						22	23	14	14	16
Follicular cyst(s)						13	16	12	20	14
Testes	(70)	(50)	(55)	(59)	(70)					
Testicular atrophy	8	12	17	13	11					
Polyarteritis	12	10	17	12	13					
Pituitary	(70)	(58)	(61)	(58)	(70)	(70)	(57)	(65)	(64)	(70)
Hyperplasia	21	13	19	13	19	22	13	9	11	18
Adrenal Cortex	(70)	(58)	(64)	(58)	(70)	(70)	(64)	(54)	(61)	(70)
Cystic degeneration	5	4	7	2	7	53	56	52	57	50
Sciatic Nerve	(70)	(3)	(45)	(46)	(70)	(70)	(43)	(42)	(43)	(70)
Swollen degenerate nerve fibers	56	30	36	31	48	42	22	24	19	44

[a]Data were extracted for study No. HST 289/8810c7, Table 12a (pp. 116-140) and Table 12b (pp. 141-161).

[b]This total includes rats found dead or sacrificed in extremis and those sacrificed after 104 weeks of treatment in both the main and satellite groups.

[c]The number of animals with blood vessels examined histologically could not be determined from the tabulated data.

[d]Numbers in parentheses are the number of animals with the specific organ examined microscopically.

The incidence of progressive glomerulonephrosis was high in all groups including controls. The severity appeared to be dose-related. The incidence of severe (marked) glomerulonephrosis was increased in both males and females receiving 75 ppm. In males, the increased incidence at 75 ppm was accounted for by rats that died. In decedents, the incidence combining both males in the main and satellite groups was 10/41, 10/43, 17/46 and 14/46 and 20/46 at 0, 3.0, 7.5, 15.0, and 75.0 ppm. The incidence in high-dose males (30/70, 43%) was reported to be higher than normally seen for historical controls. The laboratory control incidence in six studies was 70/300 (19.7%) with a range of 10 to 38%.

The incidence of aneurysms of the blood vessels was increased in the high-dose males of both the satellite (6/20) and the main study (13/50) when compared with controls (1/20 and 9/50). The percent incidence (27%) in the combined high-dose males was higher than normally found in historical controls (10%, range in five studies 4-18%). Other nonneoplastic findings were considered with the normal range of background. The most common findings were myocardial scarring, hemosiderosis of the spleen, cystic degeneration of the adrenal cortex, and peripheral nerve fiber degeneration.

2) Neoplastic: Table 10 summarize selected neoplastic lesions. No effect of dosing on the incidence or type of tumors was seen. The most commonly occurring neoplasms were adenomas of the anterior pituitary in males and females and fibroadenomas and adenocarcinomas of the mammary glands in females.

D. STUDY AUTHORS' CONCLUSIONS:

The study authors concluded that endosulfan was not oncogenic in this study. The kidneys were the primary target organ for toxicity indicated by an increase in the incidence of marked progressive glomerulonephrosis in the high-dose male rats that died. An increased incidence of blood vessel aneurysms in males receiving 75.0 ppm may have been associated with the renal lesions. Mean body weight gains were significantly decreased ($p < 0.01$) when compared to controls in males and females receiving 75.0 ppm and were considered to be of toxicological importance. No treatment-related effects were noted in other measured in-life parameters and no notable effects were observed in organ weight data and neoplastic

TABLE 10. Selected Neoplastic Lesions in Rats of Main Study Fed Endosulfan for 2 Years[a,b]

Organ/Neoplasm	Dietary Level (ppm)									
	Males					Females				
	0	3.0	7.5	15.0	75.0	0	3.0	7.5	15.0	75.0
Liver	(70)	(70)	(70)	(70)	(70)	(70)	(70)	(70)	(70)	(70)
Hepatocellular carcinoma	1	1	2	2	2	0	0	0	0	1
Hepatocellular adenoma	0	1	0	2	1	0	0	0	0	0
Hemangiosarcoma	0	0	1	0	0	0	0	1	0	0
Kidneys	(70)	(70)	(70)	(70)	(70)	(70)	(70)	(70)	(70)	(70)
Renal carcinoma	0	1	1	0	0	0	0	1	0	0
Renal liposarcinoma	2	0	2	0	1	1	0	0	0	0
Pancreas	(70)	(58)	(63)	(57)	(70)	(70)	(48)	(47)	(51)	(70)
Islet cell carcinoma	5	10	3	7	6	0	2	1	1	1
Islet cell carcinoma	11	9	11	6	8	4	6	2	7	6
Thyroid gland	(70)	(48)	(51)	(47)	(70)	(70)	(46)	(44)	(45)	(70)
C-cell carcinomas	5	3	3	1	3	1	4	1	2	0
C-cell adenomas	0	0	1	0	0	0	1	0	0	0
Follicular carcinoma	2	4	0	2	2	1	2	0	0	0
Follicular adenomas	1	3	4	5	5	0	0	1	0	1
Adrenal gland	(70)	(60)	(64)	(52)	(70)	(70)	(70)	(70)	(69)	(70)
Pheochromocytoma B	3	2	3	2	5	1	0	0	0	1
Pheochromocytoma M	0	0	2	1	1	0	0	0	0	0
Pituitary gland (anterior)	(70)	(57)	(61)	(58)	(70)	(70)	(67)	(65)	(64)	(70)
Adenoma	29	29	22	29	34	40	49	53	47	57
Carcinoma	2	0	0	0	0	3	1	0	2	2
Testes	(70)	(50)	(56)	(59)	(70)					
Interstitial cell tumor	0	2	3	2	3					

(continued)

TABLE 10. (continued)

Organ/Diagnosis	Dietary level (ppm)									
	Males					Females				
	0	3.0	7.5	15.0	75.0	0	3.0	7.5	15.0	75.0
Skin/subcutis	(70)	(54)	(57)	(60)	(70)	(70)	(48)	(47)	(49)	(70)
Fibroma	0	0	2	3	0	0	0	0	1	0
Fibroma, dermal	5	3	2	2	1	0	0	0	0	0
Keratoacanthoma	2	6	8	5	2	0	1	0	0	0
	1	0	0	0	2	0	0	0	0	0
Mammary fibroadenoma	2	2	3	1	0	42	52	48	41	45
Mammary adenocarcinoma	0	0	1	2	0	19	16	20	21	24

[a]Includes animals in both the main and satellite groups and combines data for animals that died or were sacrificed in extremis and those sacrificed at study termination. Data were extracted from Tables 11a and 11b of the study (pp. 98-111). Neoplasms occurring at an incidence of 1.5% or less in all groups were not necessarily included.

[b]The numbers in parentheses denote the number of rats with a specific tissue or organ examined microscopically.

findings. It was concluded that the LOEL was 75.0 ppm and the NOEL was 15.0 ppm for rats when endosulfan was fed for 2 years.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The protocol was complete and the conduct of the study was adequate. Reporting of data was generally adequate. However, a tabulation of animal disposition data (animal numbers at dates of death or sacrifice) was not present, and a summary of clinical signs was not presented. The individual animal pathology sheets provided data for week of death and noted clinical findings during the study and prior to death.

Mortality in the satellite groups at termination was stated in the text of the report. Scanning of the individual pathology sheets, however, indicated that deaths were recorded through week 107. It would appear that mortality at 103 weeks was not greater in the satellite groups than in the main groups. Several deaths occurred after 103 weeks when the main groups were terminated. At week 78, survival in groups of satellite males ranged between 80 to 95% and in females 65 to 85%. Only one death was related to a bleeding accident.

The reviewer checked the incidence of clinical signs in controls and high-dose males and females with particular attention to neurologic signs. Brown staining of fur, hair loss, and prominent vertebrae were frequent findings in controls and high-dose groups, as was hunched posture and pale extremities prior to death. Altered gait and hypersensitivity were seen in about 10 to 15% of the rats in control and high-dose groups; tremors, circular motion, and limb function loss were infrequent. No adverse findings were increased by dosing.

An MTD for the study was achieved based on decreased mean body weights and body weight gains in males and females receiving 75.0 ppm. Although there were some decreases in food consumption in males at 15.0 and 75 ppm (weeks 65 to 104 only), we do not assess these to be of biological importance; likewise, the decreased food conversion in dosed males from weeks 1 to 26 is not considered of importance since there were both increases (weeks 1 to 4 and 13 to 16) and decreases (weeks 5 to 8 and 9 to 12) at subset intervals and the effects were marginal.

We agree with the study authors' conclusions that there were no effects of biological importance on clinical laboratory parameters; organ weight data were not affected by dosing. Gross findings in the kidney and blood vessels were supported by histologic changes. Aneurysms in blood vessels were increased in high-dose males when compared to controls; the increase was seen in both the satellite and main groups.

Although the incidence of glomerulonephrosis of the kidneys was frequent in all groups of males and females, the severity of the finding was increased with dosing, and the incidence of marked glomerulonephrosis was definitely higher in high-dose males and females than in controls. The incidence of follicular adenoma in the thyroid of dosed males was 7.1% in mid- and high-dose males when compared to 1.4% for concurrent controls. Since the control incidence normally ranges up to 5% (0 to 5%), the observed increase is not considered to be of toxicologic importance. The incidence of all other neoplasms is within the normally experienced control range in all dosed groups and there were no increases considered related to dosing.

We assess that the ~~#~~CEL for this study is 75.0 ppm, based on decreased body weight gains and an increased incidence of marked glomerulonephrosis in males and females and an increased incidence of aneurysms in males. The ~~N~~CEL is 15.0 ppm endosulfan.

EPA No.: 68D80056
DYNAMAC No.: 210-E
TASK No.: 2-10E
January 10, 1990

DATA EVALUATION RECORD

ENDOSULFAN

Chronic Toxicity Feeding Study in Dogs

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]

Date: 1/10/90

EPA No.: 68D80056
DYNAMAC No.: 210-E
TASK No.: 2-10E
January 10, 1990

DATA EVALUATION RECORD

ENDOSULFAN

Chronic Toxicity Feeding Study in Dogs

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DATA EVALUATION RECORD

GUIDELINE §83-1

STUDY TYPE: Chronic Toxicity Feeding Study in Dogs.

MRID NUMBER: 410995-01.

TEST MATERIAL: Endosulfan-technical.

SYNONYMS: 6, 7, 8, 9, 10, 10-Hexachloro-1,5,5a,6,9,9a hexahydro-6,9 methano-2,4,3-b endodioxanthiepin-3-oxide.

STUDY NUMBER(S): 87.0643.

SPONSOR: Hoechst Celanese Corporation Somerville, NJ 08876.

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

TITLE OF REPORT: Endosulfan-Substance Technical (Code: HOE 002671 OI ZD96 0002) Testing for Toxicity by Repeated Oral Administration (1-Year Feeding Study) to Beagle Dogs.

AUTHOR(S): R. Brunk.

REPORT ISSUED: January 20, 1989.

CONCLUSIONS: Endosulfan was fed in the diet to beagle dogs for 1 year at levels of 0, 3, 10, and 30 ppm. An additional group of six dogs/sex received 30 ppm for 54 days when the dose was increased to 45 ppm and continued at that level until a final increase to 60 ppm at 106 days. At the highest dose, severe nervous symptoms developed. A loss or weakening of placing and righting reactions was seen and substantial weight loss resulted. This group was sacrificed at 146 to 147 days owing to poor overall condition; one male had been sacrificed at 126 days. In dogs receiving 30 ppm, there were decreased weight gains in males and tonic contractions of the muscles of the abdomen and chaps a few hours after feeding in both sexes; one male was sacrificed humanely at 39 weeks. There were no effects of dosing on clinical laboratory findings. Organ weights and gross findings were not affected by dosing. The sacrificed dog dosed at 30 ppm had gross and histologic changes in the lungs. The sacrificed dog receiving 60 ppm had pulmonary edema. The histologic findings in dosed and control groups for dogs sacrificed by design were generally unremarkable and incidental. No histologic effects on the brain, spinal column, or sciatic nerve accompanied the neurologic signs. Based on decreased weight gain in males and neurologic findings in both sexes, the LOEL is 30 ppm and the NOEL is 10 ppm, which corresponds to an average daily endosulfan intake of 0.65 and 0.57 mg/kg body weight in males and females, respectively.

Classification: CORE minimum.

A. MATERIALS:

1. Test Compound: Endosulfan technical--approximately [redacted] alpha- and [redacted] beta-Endosulfan; batch No. 4039; purity: 96.5% (see Appendix 1 for impurities).

2. Test Animals: Species: Beagle dog; strain: BEAK (Hoechst); age: 6 months; weight: 7.9-13.3 kg--females; 8.3-14.0 kg--males; source: Hoechst breed. The dogs were vaccinated four times for distemper, hepatitis, leptospirosis, rabies, and parvovirosis.

B. STUDY DESIGN:

1. Animal Assignment: Dogs were assigned randomly to the following groups according to body weight:

Test Group	Dose in Diet (ppm)	Main Study (54 weeks) Males	Females
1 Control	0	6	6
2 Low (LDT)	3	6	6
3 Mid (MDT)	10	6	6
4 High (HDT)	30	6	6
5 Highest[a]	30/45/60	6	6

[a]This group was started approximately 3 months after the other groups. The dietary level was increased from 30 to 45 ppm after 54 days of dosing; it was further adjusted to 60 ppm after 106 days. The group was terminated after 146 or 147 days.

2. Rationale for Dose Selection: The dose levels were based on a 14-day feeding study using one male and one female per dietary level. There were no effects at 3 ppm. At 30 ppm, delayed eating and leftover food resulted; in the female, vomiting and staggered gait were observed. Treatment with 60 ppm for 2 days resulted in food refusal by both the male and female, and vomiting by the female.

   Dogs were housed in separate kennels (0.95 x 1.0 m), and dogs of the same group and sex had access to an outdoor exercise area.

3. Diet Preparation: Premixes of the test compound in corn meal were supplied to the testing laboratory; batches of 400 g at levels of 3000, 10,000 and 30,000 ppm test compound were received monthly and were analyzed for test compound content. Cornmeal premixes (0.8 to 1.0 g) were mixed with the individual dietary rations daily, and the dry diet was made up with two parts of water. The controls received cornmeal mixed in the same proportions in their diet.

   Results: Stability of premixes was analyzed at 1, 3, 6, and 9 months. Premixes were completely stable for 30 days. The mean analyzed concentrations (± S.D.) of test compound in premixes were 97 ± 10.4, 102 ± 6, and 90 ± 6% of target at 3000, 10,000, and 30,000 ppm, respectively. The test compound was found to be completely stable in the moist diets for 24 hours (measured once for a 30-ppm diet).

4. Food and Water Consumption: Animals received Vipromix in daily portions of 1000 g for males and 800 g for females. Water was available ad libitum.

5. Statistics: The following parameters were evaluated statistically: hematology, clinical chemistry, urinary volume, specific gravity and pH, absolute and relative organ weights, and final body weights. Data for males and females were combined for analysis of hematology, organ weight data, and several clinical chemistry parameters. Data for calcium, bilirubin, urea, creatinine kinase, aspartic aminotransferase, alanine aminotransferase, and alkaline phosphatase were separated by sex for analysis. Dunnett's test or the method of Sidak was used for parametric data, and the distributed free method of Nemenyi-Dunnett was used for other data.

5. Quality Assurance: A quality assurance statement was signed and data March 28, 1989. A Good Laboratory Practice Compliance statement was also present.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected at least twice daily for mortality. General health checks were performed daily. Dogs were observed 2 hours after feeding to identify animals with delayed food consumption.

Results: No spontaneous deaths occurred. A male receiving 30 ppm (animal No. 4141) was sacrificed humanely on day 276; it was in generally poor condition and had edematous swelling of the knee joints. All dogs in the highest dose group (60 ppm) were sacrificed on days 146 and 147 because of marked and increasing nervous symptoms. One of the males in this group (animal No. 4298) showed poor general condition and was sacrificed in extremis on day 126.

Beginning on day 126, the dogs were fed early in the morning so that signs of toxicity occurring several hours after feeding could be observed. Violent contraction of the abdominal muscles and upper abdomen and a convulsive movement of the chap muscles was observed 2.5 to 6 hours after feeding in three males and two females receiving 30 ppm; no vomiting occurred. All dogs in group 5 had pronounced nervous symptoms after dosing with 60 ppm. Extreme sensitivity to noise and optical stimuli and tonic contractions of the muscles of the extremities, face, and

chaps were observed. In some cases, these reactions were violent. There were no toxic signs in dogs in the 3- and 10-ppm dose groups.

2. Body Weight: Body weights were recorded weekly and at sacrifice.

   Results: Table 1 summarizes data on mean body weights and body weight gains. Mean body weights were similar in controls and groups dosed at 3 or 10 ppm. Mean body weights in males receiving 30 ppm showed a downward trend from week 46 to termination. The overall weight gain in males receiving 30 ppm (to week 54) was 30% lower than controls. In the groups receiving 30/45/60 ppm, the weights were similar to controls and other groups for the first 12 weeks. When the dose was increased to 60 ppm (week 15), there was a decreased weight gain which was most notable in the last few weeks before termination. At 60 ppm, the mean weight loss between weeks 15 and 21 was 0.36 and 0.45 kg for males and females, respectively.

3. Food Consumption and Compound Intake: Dogs were observed 2 hours after food was offered and those dogs with food remaining were recorded. The diet remaining the following morning was weighed.

   Results: Food consumption was affected only in some dogs in groups 4 and 5. Consumption was delayed in half of the dogs in group 5 during the first 2 weeks and in three males and three females in group 4 between weeks 1 to 7. One male (Animal No. 4141) receiving 30 ppm (group 4) refused food for 5 days prior to death. One male in group 5 (Animal No. 4296) had reduced food consumption from weeks 16 to 21; its overall food consumption was reduced 10% when compared to controls. Two additional males in group 5 had reduced food consumption during the week prior to termination of the group. The report did not tabulate data for compound intake on food efficiency.

4. Ophthalmology: Ophthalmological examinations were performed prior to initiation and termination on all dogs and every 3 months in controls and in the groups receiving 30 ppm or 30/45/60 ppm. Pupils were dilated and examinations made using direct ophthalmoscopy (fundus camera) and a slit lamp microscope.

TABLE 1. Mean Body Weights and Weight Gains (kg ± S.D.) at Selected Intervals in Dogs Fed Endosulfan[a]

Dose Group (ppm)	Mean Weight (kg ± S.D.) at Week: 0	8	21	44	54	Weight Gain (kg ± S.D.) Between Weeks: 0-21	0-54
				Males			
0	11.0 ± 2.14	13.3 ± 1.70	14.5 ± 1.15	15.6 ± 1.05	15.6 ± 1.02	3.60 ± 1.39	4.68 ± 1.39
3	11.1 ± 1.88	12.3 ± 1.51	13.5 ± 1.46	14.9 ± 1.34	14.8 ± 1.39	2.45 ± 0.73	3.68 ± 0.90
10	11.2 ± 1.65	12.9 ± 1.11	14.2 ± 0.93	15.5 ± 0.76	15.3 ± 0.79	3.12 ± 0.78	4.16 ± 1.02
30	11.1 ± 1.34	13.1 ± 0.84	14.5 ± 0.75	15.4 ± 0.89	14.3 ± 0.67	3.48 ± 0.96	3.33 ± 1.29
30/45/60[b]	12.9 ± 0.82	14.0 ± 1.20	14.8 ± 1.11	--	--	1.87 ± 0.66	--
				Females			
0	10.6 ± 1.77	11.6 ± 1.21	12.2 ± 1.13	12.7 ± 1.02	12.8 ± 1.17	1.62 ± 0.92	2.22 ± 1.10
3	10.5 ± 1.27	11.5 ± 1.07	12.1 ± 0.97	13.1 ± 1.14	13.5 ± 1.30	1.60 ± 0.55	2.98 ± 0.76
10	10.6 ± 1.05	11.8 ± 0.74	12.5 ± 0.57	13.7 ± 0.61	14.0 ± 0.82	2.08 ± 0.72	3.43 ± 1.14
30	10.6 ± 0.86	11.5 ± 0.57	11.9 ± 0.57	12.8 ± 1.07	12.8 ± 1.17	1.30 ± 0.53	2.20 ± 1.02
30/45/60[b]	11.7 ± 0.94	12.4 ± 0.72	12.8 ± 1.28	--	--	1.17 ± 0.67	--

[a]Means and standard deviations were calculated by the reviewers.

[b]Animals were sacrificed at 21 weeks.

Results: There were no compound-related effects on ophthalmic findings. There were minor changes prior to initiation in three males assigned to group 5 (small glittering spots on the lens border of two males and clear lens suture lines on one male), but these findings were absent from the dogs at 6 weeks and 3 months. No other abnormal findings were noted.

5. Neurological Examinations: Examinations were conducted on all dogs prior to initiation of dosing, at 6 weeks (groups 4 and 5 only), and every 3 months in controls and groups 4 and 5. Flexor, patellar, oral, cutaneous, corneal, and blink reflexes were checked. The extensor thrust was evaluated; visual and tactile stimulated placing reactions and righting reactions were also evaluated. Hearing tests were conducted at the same intervals.

Results: The male receiving 60 ppm that was sacrificed moribund at day 126 (Animal No. 4298) had a deficit in placing and righting reactions after tactile stimuli, and another male in the same group had a similar finding when examined prior to termination (day 146). Four of six females receiving the 60-ppm dose also had a deficit in placing or righting reaction at the examination prior to termination. Reactions in these females were not obtained by tactile stimuli and they were delayed after visual stimuli. There were no effects of dosing on other postural or reflex actions. No neurological effects were observed in dogs dosed at 3, 10, or 30 ppm.

6. Hematology and Clinical Chemistry: Blood was collected from the vena cephalica antebrachii of fasted animals prior to study initiation, after 6 weeks and every 3 months (interim values), and before termination. Hematology and clinical analyses were performed on all dogs. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)†
X Hemoglobin (HGB)†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
X Platelet count†
X Reticulocyte count (RETIC)
Red cell morphology
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Coagulation: thromboplastin time (PT)
X Heinz bodies
X Methemoglobin (study termination only)

†Recommended by Subdivision F (October 1982) Guidelines.

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Results: There were no treatment-related or dose-related effects on hematology parameters. The hematocrit value and erythrocyte count were slightly increased in males and females dosed at 30/45/60 ppm at 6 weeks and 3 months when compared to controls or compared to pretest values. These increases were reported to be associated with decreased fluid intake. All hematology values were generally within the normal range.

b. Clinical Chemistry

	Electrolytes
X	Calcium[†]
X	Chloride[†]
X	Magnesium[†a]
X	Phosphorus[†]
X	Potassium[†]
X	Sodium[†]

	Enzymes
X	Alkaline phosphatase (ALP)
X	Cholinesterase (CHE)[b]
X	Creatinine phosphokinase[†]
X	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (SGPT)[†]
X	Serum aspartate amino-transferase (SGOT)[†]
X	Gamma glutamyltransferase (GGT)

	Other
X	Albumin[†]
X	Albumin/globulin ratio
X	Blood creatinine[†]
X	Blood urea nitrogen[†]
X	Cholesterol[†]
X	Globulins
X	Glucose[†]
X	Total bilirubin[†]
X	Direct bilirubin
X	Total protein[†]
X	Triglycerides
X	Iron
X	Total Lipids[a]
X	Electrophoresis
X	Uric Acid

Hepatic and renal function tests were performed on all dogs prior to dosing and in groups receiving 30 ppm (Groups 4 and 5) at 6 weeks, and in controls and groups 4 and 5 at 3-month intervals. Bromsulphthalein was measured at 2 and 45 minutes after injection, and phenosulfothalein was measured at 10 and 30 minutes after injection.

[†]Recommended by Subdivision F (October 1982) Guidelines.

[a]Terminal value only.

[b]Serum and erythrocyte-CHE activity were determined twice prior to initiation, 24 hours after one and seven treatments, after 6 weeks, every 3 months, and prior to termination. Brain-CHE was determined at group termination.

Results: The study authors reported that no compound-related effects on clinical chemistry parameters or cholinesterase activity were found. Serum alkaline phosphatase (AP) levels in control and dosed groups decreased markedly compared to the pretest values with time (Table 2). In control males, the activity at 6 months was 33% of pretest and in females it was 54% of pretest. When pairwise comparisons were made between control and dosed groups, there was a significant increase ($p < 0.05$) in AP activity in males and females receiving 30 ppm at all intervals beginning at 1.5 months and in both sexes receiving 10 ppm at several intervals. There were no effects on any other serum enzymes.

There was no compound-related depression of plasma (PCHE) or erythrocyte cholinesterase activity (RCHE). The RCHE activity at day 7 of dosing was about twice the value of control activity in group 5 males and females receiving 30 ppm; however, there was no comparable effect on dogs in group 4 that were also receiving 30 ppm. The high values are considered anomalous and of no toxicologic importance. The study authors considered that there was no effect of dosing on brain cholinesterase activity. However, the data do not support any conclusion (see Reviewers Discussion, Section E).

Hepatic function testing did not indicate any impairment in bromosulfothalein retention caused by dosing. Renal function testing did not show any delayed excretion of phenosulfophthalein caused by endosulfan administration.

6. Urinalysis: Urine was collected from all animals at quarterly intervals as well as at week 6. The CHECKED (X) parameters were examined:

X	Appearance†	X	Glucose†
X	Volume†	X	Ketones
X	Specific Gravity†	X	Bilirubin†
X	pH	X	Blood†
X	Sediment (microscopic)†		Nitrate
X	Protein†	X	Urobilinogen
X	Color		

Results: Urinary parameters were similar in control and dosed dogs.

†Recommended by Subdivision F (October 1982) Guidelines.

TABLE 2. Mean Serum Alkaline Phosphatase Levels (U/L) in Dogs Fed Endosulfan for 1 Year

Interval (months)	Dietary Level (ppm)				
	0	3	10	30	30/45/60[a]
			Males		
0	220	224	230	235	193
1.5	137	151	156*	214*	205
3	118	139	157*	196*	199
6	73	98	117*	136*	--
9	75	91	92	141*	--
12	61	80	99*	146*	--
			Females		
0	182	164	224	205	195
1.5	135	128	175*	245*	201
3	131	125	152*	201*	212
6	99	87	117	168*	--
9	117	108	135	199*	--
12	95	99	129	183*	--

[a]Dogs in this group were sacrificed after 146 days; statistical notations were not provided for this group.

*Significantly different from control value, $p < 0.05$.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

Digestive System	**Cardiovasc./Hemat.**	**Neurologic**
X Salivary glands†	X Aorta†	XX Brain
X Esophagus†	XX Heart†	X Peripheral nerve (sciatic nerve)†
X Stomach†	X Bone marrow†	X Spinal cord (3 levels)
X Duodenum†	X Lymph nodes† (cervical iliac)	XX Pituitary†
X Jejunum†	XX Spleen	X Eyes (optic nerve)†
X Ileum†	XX Thymus	
X Cecum†		
X Colon†	**Urogenital**	**Glandular**
X Rectum	XX Kidneys†	XX Adrenals†
XX Liver†	X Urinary bladder†	Lacrimal gland
X Gallbladder†	XX Testes†	X Mammary gland†
X Pancreas†	XX Epididymides	XX Thyroids†
	XX Prostate	XX Parathyroids†
Respiratory	Seminal vesicle	Harderian glands
X Trachea†	XX Ovaries	X Tonsils
XX Lung†	XX Uterus	
		Other
		X Bone (sternum and femur)†
		X Skeletal muscle†
		X Skin
		X All gross lesions and masses
		X Nasal septum
		X Diaphragmatic muscles

†Recommended by Subdivision F (October 1982) Guidelines.

Results:

a. Organ Weights: Mean organ weights were similar in control and dosed dogs and were within the normal range. A male that had received 60 ppm and was sacrificed in poor condition on day 126 (Animal No. 4298) had an increased lung weight, which was associated with pulmonary edema and pneumonia.

b. Gross Pathology: No gross abnormalities were found in dogs that were sacrificed at study termination at 12 months; in addition, there were no gross abnormalities in the dogs in group 5 that were sacrificed on days 146 and 147 after severe neurological disturbances caused by dosing at 60 ppm. The male receiving 30 ppm (Animal No. group 4) that was sacrificed in extremis on day 126 (Animal No. 4141) had connective tissue induration in the hilum of the lung and enlarged hilar lymph nodes. The male of group 5 that was sacrificed after receiving 60 ppm had pulmonary edema and hyperdistention of the lungs. All other dogs had only incidental findings.

c. Microscopic Pathology: Histologic examination of the male receiving 30 ppm (group 4) that was sacrificed on day 270 confirmed the gross findings. There was severe purulent mediastinitis involving the pleura and parenchyma of the lungs, which extended to the vicinity of the thymus. The group 5 male sacrificed at day 126 had severe confluent aspiration pneumonia. The finding may have been secondary to the neurological symptoms. There were no histologic findings in the cerebrum, cerebellum, brainstem, medulla oblongata, or the three levels of the spinal cord examined in dogs in the control or dosed groups. Table 3 summarizes lesions that were frequent in dogs. The grade of most lesions was minimal or slight (grade 1 or 2). There were no increases in any histologic findings considered related to dosing. No neoplasms were noted.

D. STUDY AUTHOR'S CONCLUSIONS:

All dogs at the highest dose (30/45/60 ppm) had to be killed prematurely (days 126, 147-148); a male receiving 30 ppm was killed intercurrently on day 276. All other dogs survived to 12-months. There were no compound-related changes at 3.0 or 10.0 ppm. Average body weight gains were lower in males receiving 30 ppm, but there were only slight depressions of food consumption. Administration of 30 ppm resulted in tonic contractions of the abdominal and chap muscles. There were no effects on clinical laboratory findings or on cholinesterase

TABLE 3. Histopathologic Findings in Dogs Fed Endosulfan for 1 Year[a]

Organ/Findings	Dietary Level (ppm)									
	Males					Females				
	0	3	10	30	30-60	0	3	10	30	30-60
Kidneys										
Frequent concretements	4	3	3	2	4	4	4	1	3	4
Liver										
Kupffer cell nodules	0	2	0	1	1	2	3	1	3	2
Lungs										
Peribronchial round cells	0	2	0	0	2	2	1	1	0	0
Testes										
Focular tubular atrophy	0	1	4	0	1					
Uterus										
Proliferation of endometrium						3	4	1	3	1
Lymph nodes/cervical										
Hyperplasia (T region)	2	2	0	0	1	3	4	1	1	4

[a]Six dogs/sex/group were examined histologically.

activity. At the highest dosage (30/45/60 ppm), there were impairment of the general condition and decreased body weight gains from 12 weeks onward. Administration of 60 ppm resulted in nervous symptoms and a weakening of abolition of the placing and righting reactions, indicating effects on the central nervous system. Erythrocyte counts and hematocrit values were increased compared to controls and the pretreatment values in dogs receiving 38/45/60 ppm; this effect was attributed to reduced water consumption. There were no effects on other clinical laboratory findings or on histopathologic findings. The NOEL was approximately 10 ppm, corresponding to a mean substance intake of 0.65 mg/kg/day for males and 0.57 mg/kg/day for females.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct of the study and reporting of the results were adequate. Individual data for all parameters were presented. Summary tabulation data for clinical laboratory data were complete, but the format for presentation made intergroup comparisons difficult. The summary tabulation for hematology and clinical chemistry data (means and standard deviations) was 153 pages long, and statistical notations were tabulated separately (31 pages). Normal testing ranges were not provided and would have been useful in evaluating some of the clinical data. The levels of alkaline phosphatase at pretest (see Table 2) were considerably higher than we have experienced. Data from another laboratory gave mean values of 62, 45, and 26 IU/L for dogs 0-6, 7-12, and >12 months of age. The study authors do not consider the significant increases in alkaline phosphatase when dogs receiving 10 or 30 ppm are compared to respective controls to be toxicologically important. However, we do not feel we can judge the importance of the changes without laboratory reference data. High values for erythrocyte cholinesterase activity in both males and females of group 5 at day 7 are considered by the reviewers to be due to technical error. The mean values (504, U/L males; 597 U/L, females) are approximately twice the value of group 4 dogs at the same dose at day 7. Values at other intervals for the group 5 dogs were similar to values in other groups and did not differ from controls. We agree with the study authors conclusion that was no effect of dosing on cholinesterase activity of plasma or erythrocytes. However, it is our assessment that data on brain cholinesterase activity do not support any conclusion. Table 4 presents mean data. There was a large range of values for individual dogs in most groups. It cannot be determined if this is due to technical errors or normally experienced variability. Ranges of normal values for the testing laboratory may clarify this.

We agree with the study author's conclusions that the LOEL is 30 ppm based on decreased weight gain and neurologic symptoms, and that the NOEL is 10 ppm.

TABLE 4. Mean Brain Cholinesterase Activity (U/kg ± S.D.) for Dogs Fed Endosulfan for 1 Year[a]

Dose Group	Males	Females
0	1268 ± 708	578 ± 100
3	862 ± 654	1729 ± 1572
10	1031 ± 706	738 ± 524
30	563 ± 143(5)	3710 ± 2389
30/45/60[b]	589 ± 53(5)	815 ± 528

[a]Unless otherwise noted, mean values represent results for six dogs killed after scheduled sacrifice.

[b]Results for five males and six females killed on day 146 (early group termination); no data were available for one dog killed for humane reasons on day 126.

CBI APPENDIX

Analysis of Impurities

Endosulfan toxicology review

Page _____ is not included in this copy.

Pages 67 through 68 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

007937

EPA No.: 68D80056
DYNAMAC No.: 210-A
TASK No.: 2-10A
January 23, 1990

DATA EVALUATION RECORD

ENDOSULFAN

Subchronic Dermal Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 1/23/92

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EPA No.: 68D80056
DYNAMAC No.: 210-A
TASK No.: 2-10A
January 23, 1990

DATA EVALUATION RECORD

ENDOSULFAN

Subchronic Dermal Toxicity Study in Rats

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DATA EVALUATION RECORD

GUIDELINE § 82-2

STUDY TYPE: Subchronic dermal toxicity study in rats.

MRID NUMBER: 410485-05.

TEST MATERIAL: Endosulfan Emulsifiable Concentrate; Code No. HOE 002671 OI EC34 A101.

SYNONYM(S): 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathi epin-3-oxide; Thiodan; Cyclodan; HOE 2671.

STUDY NUMBER(S): 094590.

SPONSOR: Hoechst Celanese Corporation, Somerville, NJ.

TESTING FACILITY: Research and Consulting Co. AG, Itingen, Switzerland.

TITLE OF REPORT: Subchronic (4-week) Repeated Dose Dermal Toxicity Study in Rats.

AUTHOR(S): Thevanaz, P., Leutkemeier, H., Chevalier, H. J., Vogel, W., and Terrier, C.

REPORT ISSUED: October 4, 1988.

CONCLUSIONS: Groups of 15 male and 15 female rats were dosed with dermal applications of endosulfan for 6 hours per day, 5 days per week for a total of 21-22 applications. Dose levels were 27, 54, or 81 mg/kg for males and 9, 12, 18, or 36 mg/kg for females. Additional groups of 15 males and 15 females were treated with formulation base administered in the vehicle or with the vehicle alone. Ten rats/sex/group were sacrificed at the end of the treatment period. The remaining five/sex/group were sacrificed after a 4-week recovery period. Marginal to slight erythema and marginal edema were exhibited in rats dosed dermally with endosulfan; erythema persisted through the first week of recovery. Rats dosed with the formulation base exhibited minimal to moderate erythema and minimal to slight edema; erythema persisted through the recovery period. At necropsy, sores and red discoloration were observed at the treated skin site. Hyperkeratosis and inflammation of the skin were evident upon microscopic examination in all groups including the vehicle control. The severity and incidence of these findings were more prominent in rats treated with the formulation base. The presence of these findings in controls and endosulfan-treated rats were considered to reflect mechanical irritation resulting from hair clipping.

Clinical signs indicative of central nervous system involvement were seen in males dosed with 81 mg/kg and females dosed with 12, 18, or 36 mg endosulfan/kg. These signs were transient, generally occurring within hour of dermal application and disappearing within 30 minutes of onset. Four females dosed dermally with 36 mg/kg, one female dosed with 18 mg/kg, and one female dosed with 12 mg/kg died during the study period; the death of two additional animals (one male, one female) were considered to be unrelated to dosing. Body weights of males dosed with 54 and 81 mg/kg were slightly depressed throughout the dosing period; body weights of other dosed animals were similar to those of concurrent controls.

Levels of alkaline phosphatase were increased in females dosed with 36 mg/kg, while albumin levels of females dosed with 18 and 36 mg/kg were decreased following dosing. Plasma cholinesterase activities of females dosed with 12, 18, and 36 mg/kg were depressed following dosing. No effects on erythrocyte or brain cholinesterase were exhibited. There were no changes in organ weights or systemic pathology that were considered to be a result of dosing. Based on mortality and clinical signs indicative of central nervous system involvement, the LOEL for systemic toxicity is 81 mg/kg in males and 12 mg/kg in females, and the NOEL is 54 mg/kg in males and 9 mg/kg in females. The formulation base was more toxic dermally than treatment with endosulfan.

Classification: CORE Supplementary. The systemic LOEL and NOEL were based in part on the clinical signs of central nervous system toxicity seen in animals following dosing. However, no quantitative data for these effects were included in the laboratory's report; therefore, it is not possible to adequately

evaluate the reported conclusions. This study will be upgraded upon submission and satisfactory review of these data.

A. MATERIALS:

1. Test Compound: Endosulfan emulsifiable concentrate (HOE 002671 OI EC34 A101); description: brown liquid; batch No.: PL 84/167; purity: 33.3% active ingredient.

2. Test Animals: Species: rat; strain: Wistar; age: 10 weeks; weight: males--214-266 g, females--200-270 g; source: KFM Kleinterfarm Madoerin AG, CH 4414, Fuellinsdorf, Switzerland.

B. STUDY DESIGN:

1. Animal Assignment: Following a 6-day acclimation period, animals were assigned randomly to the following test groups:

Test Group	Applied Substance	Dermal Dose (mg/kg)	Main Group (4 weeks)		Recovery Group (4 weeks)	
			Males	Females	Males	Females
1	Vehicle[a]	0	10	10	5	5
2	Formulation base[b]	0	10	10	5	5
3	Endosulfan	27/9[c]	10	10	5	5
4	Endosulfan	54/12[c]	10	10	5	5
5	Endosulfan	81/18[c]	10	10	5	5
6	Endosulfan	-/36[c]	--	10	--	5

[a] Vehicle: aqueous 4% carboxymethylcellulose (CMC) solution.

[b] Formulation base: HOE 002671 OI EC00 A302 administered in aqueous 4% CMC solution.

[c] Male dose/female dose; group 6, females only.

Dose levels were determined based on a previously conducted dermal study (Hoechst project No. 84'0284) that yielded a NOEL of 48 mg/kg in male rats and 9 mg/kg in female rats. A lethal threshold dose of 500 to 1000 mg/kg in males and 200 mg/kg in females resulted from preliminary testing for acute dermal toxicity (RCC Project No. 095242). No further information was reported.

2. Test Article Preparation: Endosulfan emulsifiable concentrate or the formulation base was combined with the vehicle (distilled water containing 4% carboxymethylcellulose) on a w/w basis; the mixtures were prepared daily prior to application. The stability of the test compound and formulation base in the vehicle was analyzed at study initiation; concentration and homogeneity analyses were performed during study weeks 1 and 4.

   The mean test concentrations varied within a range of 83.1% to 104.6% of the nominal concentration with the exception of one sample (81 mg/kg) with a mean concentration of 68.3%. Homogeneity was within ±10% of nominal with the exception of one sample (81 mg/kg), which was within ±70%. The study author reported that the unacceptable results of this sample analysis were due to error during analysis and not to sample preparation. The test compound was stable in the vehicle for a period of 2 hours.

3. Preparation of Animal Skin and Method of Application: The hair was clipped from the animals' backs weekly throughout the study. The test material, formulation base, or vehicle was applied evenly on the shaved skin at a dose volume of 2 mL/kg body weight over an area of approximately 10% of the total body surface area. The treated area was covered with a semi-occlusive dressing and held in place with an elastic adhesive bandage. After a 6-hour exposure period, excess test material was removed from the application site with lukewarm water. Doses were administered on a 5-day per week schedule for a total of 21-22 applications over a 29- to 30-day period. At the end of the treatment period, animals assigned to the main study groups (10/sex/group) were sacrificed. Animals assigned to the recovery groups (5/sex/group) were sacrificed after a 4-week recovery period.

4. Food and Water Consumption: Animals received food (pelleted Standard Kliba No. 343 rat maintenance food) and tap water ad libitum.

5. Statistics: The following procedures were utilized in analyzing the numerical data:

   Body weights, food consumption, organ weights, and clinical laboratory data were analyzed using the univariate one-way analysis of variance to assess the significance of intergroup differences. If variables could be assumed to follow normal distribution, Dunnett's t-test based on a pooled variance estimate was applied for the comparison

between treated and control groups. The Steel test was applied when the data could not be assumed to follow a normal distribution. Spontaneous mortality data were analyzed by Fisher's Exact test.

Quality Assurance: A quality assurance statement was signed and dated October 7, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for mortality and morbidity and once daily for signs of systemic toxicity. The application site was examined once daily for signs of local irritation. Dermal reactions were scored using a modification of the Draize scoring system (Appendix A).

Results: Mortality data are summarized in Table 1. No treatment-related mortality occurred in any of the male test groups. One control male died following anesthesia for blood collection at the end of the recovery period. Among the females, the deaths of six dosed females (one dosed with 12 mg/kg, one dosed with 18 mg/kg, and four dosed with 36 mg/kg) during treatment weeks 1, 2, and 4 were attributed to dosing. The incidence of mortality in high-dose females (36 mg/kg) was statistically significant ($p < 0.05$). The death of one additional female dosed with 18 mg/kg during week 4 of the dosing period was attributed to blood collection procedures; this death was not considered to be related to dosing. Clinical signs indicative of central nervous system involvement (tremor, Straub-tail, trismus, saltatory spasms, extension spasms, and tetanoid spasms) were exhibited during dosing in males administered 81 mg/kg and females administered 18 and 36 mg/kg, with isolated incidences in females administered 12 mg endosulfan/kg. The signs were transient, occurring within in 1 hour of dermal application and disappearing within 30 minutes of onset. There were no quantitative data included in the report to support the incidence of neurological signs. No other signs of systemic toxicity were seen during the study. Squealing was heard from all dosed animals during the daily dosing periods.

Minimal to moderate erythema was observed during the dosing and recovery periods in animals treated with the formulation base (Appendix B). Mean erythema increased in incidence and intensity in these animals toward the end of dosing and was accompanied by slight edema from week 3 of the treatment period through week 1 of recovery. The erythema exhibited by the animals decreased from moderate to marginal during the last 3 weeks of the recovery period. Marginal to slight erythema was seen in males administered

TABLE 1. Mortality Incidence in Rats Treated Dermally With Endosulfan for 4 Weeks

Dose Group (mg/kg)		Main Study Groups (10/sex/group)		Recovery Study Groups (5/sex/group)	
Males	Females	Males	Females	Males	Females
0[a]	0[a]	0	0	1[d]	0
0[b]	0[b]	0	0	0	0
27	9	0	0	0	0
54	12	0	1	0	0
81	18	0	2[c]	0	0
-	36	-	4*	-	0

[a]Vehicle alone (aqueous 4% carboxymethylcellulose solution).

[b]Formulation base (HOE 002671 OI EC00 A302), administered in the vehicle.

[c]One animal from this group died following blood sampling during treatment week 4.

[d]Animal died following anesthesia for blood sampling during recovery period.

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

54 or 81 mg/kg and females administered 12, 18, or 36 mg/kg, beginning at the second week of dosing and continuing through the first week of recovery. Marginal edema was noted in males administered 54 and 81 mg/kg and in females administered 12 and 18 mg/kg during the latter half of the treatment period; edema was not detectable during recovery. Erythema and crust formation were observed on the flanks of control and all dosed animals during the dosing period; these findings were reported to be due to the animals' scratching in attempts to free themselves from the bandages, and were not related to dosing.

2. Body Weights: Animals were weighed prior to the first treatment and weekly thereafter.

   Results: Body weights of males dosed with 54 and 81 mg/kg were found to be slightly (5 to 6%) depressed throughout the dosing period; significantly ($p < 0.05$) depressed (10 to 11%) body weights were found at day 21 for males dosed with 54 and 81 mg/kg and at day 29 for males dosed with 54 mg/kg (7%) (Table 2). Body weights of females dosed with 18 mg/kg were slightly depressed (3 to 6%) throughout the dosing period; body weights of these animals were significantly ($p < 0.05$) depressed (7%) at day 8. The study author considered these body weight reductions to be incidental. Mean body weights of other dosed males and females were similar to those of concurrent controls. All depressed body weights recovered following the dosing period.

3. Food Consumption: Food consumption was measured weekly during the treatment and recovery periods.

   Results: No compound-related effects on food consumption were noted during the treatment or recovery periods. Slight increases in the food consumption of dosed rats during the recovery period were considered by the study authors to be due to low mean food consumption in control animals at this time.

4. Ophthalmology: Ophthalmological examinations were not performed.

5. Hematology and Clinical Chemistry: Blood samples for hematology and clinical biochemistry were collected from the retro-orbital plexus at the end of the treatment and recovery periods. Animals were food-fasted for 15 to 18 hours prior to blood collection. Samples for plasma and erythrocyte cholinesterase activity determinations were collected from nonfasted animals on the day of sacrifice. Brain tissue for the measurement of cholinesterase activity was removed at necropsy and stored frozen until analyzed.

TABLE 2. Representative Results of Mean Body Weights (± S.D.) of Rats Administered Endosulfan Dermally for 4 Weeks[a]

Dose Group (mg/kg)	Mean Body Weights (g) at Day: Pretest	8	21/22	29
		Males		
0[b]	238.6 ± 12.0	286.1 ± 18.2	320.8 ± 26.3	326.0 ± 27.1
0[c]	241.8 ± 11.2	280.7 ± 11.2	296.2 ± 13.5	314.5 ± 15.7
27	242.7 ± 16.0	290.8 ± 21.8	312.1 ± 31.5	331.1 ± 33.0
54	232.0 ± 15.0	270.7 ± 17.4	286.5 ± 19.0*	303.0 ± 20.5*
81	236.9 ± 12.8	273.5 ± 20.5	290.4 ± 22.1*	307.7 ± 24.9
		Females		
0[b]	239.8 ± 20.4	242.6 ± 17.5	249.9 ± 17.1	251.6 ± 16.5
0[c]	241.0 ± 14.3	240.5 ± 14.5	245.0 ± 14.6	245.5 ± 13.5
9	236.6 ± 10.7	240.5 ± 12.8	248.6 ± 17.0	251.7 ± 19.9
12	235.6 ± 17.8	237.1 ± 18.2	248.9 ± 16.8	248.3 ± 21.2
18	231.7 ± 15.1	224.8 ± 13.1*	240.0 ± 11.5	244.2 ± 13.9
36	237.6 ± 15.5	235.9 ± 15.6	250.7 ± 12.4	252.5 ± 14.8

[a]Based on 15 rats/sex/dose.

[b]Vehicle alone (aqueous 4% carboxymethylcellulose solution).

[c]Formulation base administered in the vehicle.

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

The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)†
X Hemoglobin (HGB)†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
X Platelet count†
X Reticulocyte count (RETIC)
X Red cell morphology
X Leukocyte differential count†
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Coagulation: thromboplastin time (PT)†

Results: Review of hematological data at the end of the treatment and recovery periods revealed no changes of toxicological significance. Slight but significant ($p < 0.05$) changes in hematological parameters following dosing were considered to be incidental.

b. Clinical Chemistry

Electrolytes
X Calcium†
X Chloride†
Magnesium
X Phosphorus†
X Potassium†
X Sodium†

Enzymes
X Alkaline phosphatase (ALP)
X Cholinesterase
X Creatinine phosphokinase†
X Lactic acid dehydrogenase
X Serum alanine aminotransferase (ALAT; SGPT)†
X Serum aspartate aminotransferase (ASAT; SGOT)†
X Gamma glutamyltransferase (GGT)

Other
X Albumin†
X Albumin/globulin ratio (A/G ratio)
X Blood creatinine†
X Blood urea nitrogen†
X Cholesterol†
X Globulins
X Glucose†
X Total bilirubin†
Direct bilirubin
X Total protein†
X Triglycerides

Results: Selected mean clinical biochemistry data are presented in Table 3. ASAT activity was reported to be significantly ($p < 0.05$) (18%) increased in males dosed with 31 mg/kg following the dosing period; however, this increase was due to the elevated ASAT level of one outlier (animal No. 65). ALAT, triglyceride, and cholesterol

†Recommended by Subdivision F (October 1982) Guidelines for subchronic dermal toxicity studies.

TABLE 3. Selected Mean Clinical Biochemistry Parameters (± S.D.)[a] of Rats Treated Dermally With Endosulfan for 4 Weeks

Dose Group (mg/kg)	ASAT (U/L)		ALP (U/L)		Albumin (g/L)		Total Protein (g/L)		A/G Ratio	
	Postdosing	Postrecovery	Postdosing	Postrecovery	Postdosing	Postrecovery	Postdosing	Postrecovery	Postdosing	Postrecovery
Males										
0	71.3 ± 8.9	70.0 ± 5.6	93 ± 20.0	79 ± 12.0	35.3 ± 1.36	36.6 ± 0.49	72.1 ± 3.40	68.1 ± 3.10	0.96 ± 0.09	1.17 ± 0.12
0	81.5 ± 10.8	68.1 ± 3.1	97 ± 19.9	76 ± 12.1	33.7 ± 1.64*	36.4 ± 1.24	75.1 ± 3.86	67.7 ± 2.38	0.82 ± 0.06*	1.16 ± 0.08
27	81.0 ± 13.1	72.8 ± 14.1	92 ± 15.1	74 ± 13.3	34.9 ± 1.21	36.5 ± 1.49	76.0 ± 7.86	67.2 ± 2.13	0.88 ± 0.19	1.20 ± 0.16
54	78.3 ± 8.6	65.7 ± 4.9	89 ± 11.7	80 ± 7.1	34.8 ± 0.99	34.9 ± 2.27	68.9 ± 3.40	66.6 ± 3.41	1.03 ± 0.10	1.11 ± 0.09
81	84.3 ± 20.7*	68.7 ± 12.3	87 ± 11.8	82 ± 8.2	35.2 ± 1.36	35.2 ± 1.18	74.0 ± 4.08	66.2 ± 3.06	0.91 ± 0.06	1.14 ± 0.05
Females										
0	74.3 ± 13.4	66.5 ± 7.8	32 ± 9.9	26 ± 4.0	39.6 ± 1.6	41.0 ± 1.65	78.8 ± 3.50	73.2 ± 3.64	1.02 ± 0.08	1.28 ± 0.12
0	81.0 ± 14.1	63.1 ± 10.4	30 ± 7.1	26 ± 4.7	38.7 ± 3.0	39.8 ± 2.51	79.9 ± 3.33	70.7 ± 4.68	0.94 ± 0.09	1.29 ± 0.08
9	81.2 ± 14.2	62.3 ± 13.6	40 ± 18.4	31 ± 3.7	37.8 ± 2.2	37.2 ± 0.86*	77.1 ± 4.94	68.2 ± 1.50*	0.97 ± 0.11	1.20 ± 0.05
12	78.9 ± 12.7	74.0 ± 16.4	35 ± 9.7	37 ± 7.4	38.2 ± 2.3	37.4 ± 1.86*	76.3 ± 3.21	68.3 ± 0.76	1.01 ± 0.09	1.22 ± 0.14
18	79.9 ± 18.3	75.8 ± 19.0	35 ± 7.8	30 ± 10.7	37.2 ± 2.4*	38.2 ± 1.59	76.9 ± 4.57	69.1 ± 1.72	0.95 ± 0.14	1.24 ± 0.14
36	78.7 ± 13.1	63.4 ± 8.5	46 ± 15.1*	34 ± 6.8	36.5 ± 1.9*	36.0 ± 2.24*	76.9 ± 4.43	66.6 ± 1.37*	0.91 ± 0.11*	1.18 ± 0.13

[a]Standard deviations calculated by reviewers.

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

parameters were also increased substantially for this animal. When the group means for ASAT and ALAT are recalculated without the outlier, results are similar to those of the concurrent controls (see Reviewer's Discussion and Interpretation of Results). Alkaline phosphatase levels of females dosed with 36 mg/kg were significantly ($p < 0.05$) (46%) increased when compared with those of concurrent controls following the dosing period. Albumin levels of females dosed with 18 and 36 mg/kg and albumin/globulin ratios of females dosed with 36 mg/kg were slightly but significantly ($p < 0.05$) (7 to 11%) decreased following the dosing period. The study author considered these changes to be compound related. Slight decreases in albumin and total protein in dosed females following recovery were considered by the study author to be a result of impairment of hepatic function.

Cholinesterase activity data are presented in Table 4. Following dosing, plasma cholinesterase (BuCHE-PL) activities of females dosed with 12, 18, and 36 mg/kg were significantly ($p < 0.05$) depressed in a dose-related manner (78, 71, and 68% of concurrent control activity, respectively). Erythrocyte cholinesterase (RCHE) activities of males dosed with 27, 54, and 81 mg/kg were slightly but significantly ($p < 0.05$) depressed in a dose-related manner (87, 83, and 78% of concurrent control activity, respectively). The RCHE activities of females dosed with 12 and 36 mg/kg were also slightly depressed. Slight and sporadically depressed BCHE activities were exhibited in dosed males and females. The RCHE depression was considered by the study authors to be incidental and the result of normal biological variation. BCHE results were not addressed (see Reviewer's Discussion and Interpretation of Results).

6. Urinalysis: Urine was collected from fasted animals at the end of the treated and recovery periods. The CHECKED (X) parameters were examined:

	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

Results: No treatment-related changes were noted in the urinalysis data at the end of the treatment or recovery periods.

Dose Group (mg/kg/day)	BuCHE-PL[a] (umol·SH/mL)		RCHE (umol·SH/mL)		BCHE (umol·SH/g)	
	Postdosing	Postrecovery	Postdosing	Postrecovery	Postdosing	Postrecovery
			Males			
0	0.66 ± 0.06	0.61 ± 0.04	1.89 ± 0.20	1.68 ± 0.15	5.98 ± 0.50	7.66 ± 0.69
0	0.68 ± 0.07 (103)[b]	0.57 ± 0.08 (93)	1.85 ± 0.14 (98)	1.68 ± 0.13 (100)	6.13 ± 0.25 (103)	7.80 ± 0.49 (102)
27	0.70 ± 0.09 (106)	0.70 ± 0.08 (115)	1.64 ± 0.15* (87)	1.74 ± 0.18 (104)	6.02 ± 0.62 (101)	7.67 ± 0.41 (100)
54	0.72 ± 0.11 (109)	0.64 ± 0.05 (105)	1.56 ± 0.19* (83)	1.60 ± 0.10 (95)	5.03 ± 0.50* (84)	8.01 ± 0.75 (105)
81	0.70 ± 0.09 (106)	0.69 ± 0.11 (113)	1.48 ± 0.12* (78)	1.77 ± 0.20 (105)	6.64 ± 0.53* (111)	8.37 ± 0.94 (109)
			Females			
0	3.07 ± 0.46	3.82 ± 0.34	2.02 ± 0.26	1.68 ± 0.14	7.63 ± 0.53	9.14 ± 0.34
0	2.58 ± 0.70 (84)	3.31 ± 0.57 (87)	1.87 ± 0.20 (93)	1.77 ± 0.09 (105)	6.91 ± 0.49* (91)	8.74 ± 0.62 (96)
9	2.56 ± 0.47 (83)	3.15 ± 0.61 (82)	1.92 ± 0.20 (95)	1.80 ± 0.23 (107)	7.32 ± 0.38 (96)	8.98 ± 0.73 (98)
12	2.40 ± 0.59* (78)	3.01 ± 1.00 (79)	1.76 ± 0.26* (87)	1.76 ± 0.13 (105)	6.73 ± 0.46* (88)	9.11 ± 0.86 (100)
18	2.19 ± 0.60* (71)	3.05 ± 0.43 (80)	1.82 ± 0.14 (90)	1.82 ± 0.07 (108)	6.95 ± 0.86* (91)	9.02 ± 0.53 (99)
36	2.08 ± 0.79* (68)	3.34 ± 0.74 (87)	1.69 ± 0.26* (84)	1.77 ± 0.11 (105)	6.65 ± 0.45* (87)	9.77 ± 0.09 (107)

[a]Abbreviations: BuCHE-PL = plasma butyrylcholinesterase; RCHE = erythrocyte acetylcholinesterase; BCHE = brain acetylcholinesterase.

[b]Numbers in parentheses represent percentage of control.

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

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subjected to gross pathological examination, and the CHECKED (X) tissues were collected and fixed in 4% neutral phosphate buffered formaldehyde solution. In addition, the (XX) organs were weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta	XX	Brain
X	Salivary glands	XX	Heart	X	Peripheral nerve (sciatic nerve)
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	XX	Spleen	X	Eyes (optic nerve)
X	Jejunum	X	Thymus		
X	Ileum				Glandular
X	Cecum		Urogenital	XX	Adrenals
X	Colon	XX	Kidneys	X	Lacrimal gland
X	Rectum	X	Urinary bladder	X	Mammary gland
XX	Liver	XX	Testes	X	Thyroids
X	Pancreas	X	Epididymides	X	Parathyroids
		X	Prostate	X	Harderian glands
	Respiratory	X	Seminal vesicle		
X	Trachea	XX	Ovaries		Other
XX	Lung	X	Uterus	X	Bone (sternum and femur)
X	Nasopharynx	X	Cervix	X	Skeletal muscle
X	Larynx	X	Vagina	X	Skin (treated and untreated)
				X	All gross lesions and masses

Sections of treated and untreated skin, brain, adrenals, heart, lung, ovaries, testes, spleen, liver, kidneys, and gross lesions from all animals were microscopically examined.

Results:

a. Organ Weights: There were no compound-related changes in organ weights. Significant increases in relative brain, liver, kidneys, adrenals, spleen, and testicular weights of males dosed with 54 and 81 mg/kg and the decreased absolute liver weight of males dosed with 54 mg/kg were the result of intergroup variation in mean body weight at the time of terminal sacrifice.

[r] Recommended by Subdivision F (October 1982) Guidelines for subchronic dermal toxicity studies.

b. Gross Pathology: Sores (one to numerous) and/or reddish discoloration at the treated skin site were observed in 5/15 males and 11/15 females dosed with the formulation dose, 1/15 males dosed with 27 mg/kg, and 2/15 males and 1/15 females dosed with 54 and 12 mg/kg, respectively. No other treatment-related gross pathologic changes were observed.

c. Microscopic Pathology:

1) Nonneoplastic: Representative nonneoplastic histopathologic findings are presented in Table 5. In rats sacrificed at the end of the dosing period, minimal to moderate hyperkeratosis of the treated skin was exhibited in 9, 8, 7, and 9 males dosed with formulation base, and 27, 54, and 81 mg/kg endosulfan, respectively, and 10, 7, 8, 10, and 10 females dosed with formulation base, and 9, 12, 18, and 36 mg/kg endosulfan, respectively, as compared with 5 concurrent male controls and 8 concurrent female controls. The incidence and severity of the hyperkeratosis were considered to be similar in all groups, except for those dosed with the formulation base. The severity of hyperkeratosis in the animals of this group was slightly increased when compared with that of the other dosed animals (mean severity grade of 2.2 and 2.4 for males and females dosed with formulation base, respectively, compared to mean severity grades of 1.3 to 1.8 in all other test groups). The incidence of minimal to moderate chronic inflammation was exhibited sporadically in dosed and control animals with the exception of those dosed with the formulation base, which exhibited an increased incidence and severity of inflammation that was usually associated with ulceration. At the end of the recovery period, slight to moderate hyperkeratosis was observed in one female in each group dosed with formulation base and 18 and 36 mg/kg endosulfan. Other microscopic lesions were found similarly in dosed and control animals, were considered to be common age- and strain-related changes, and were not considered to be compound-related.

2) Neoplastic: No neoplastic changes were noted.

TABLE 5. Representative Histopathologic Findings in Rats Treated Dermally With Endosulfan for 4 Weeks

Tissue/Finding	Dose Level (mg/kg/day) Males 0	0	27	54	81	Females 0	0	9	12	18	36
Terminal Sacrifice											
Treated skin	(10)[a]	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hyperkeratosis	5	9	8	7	9	8	10	7	8	10	10
Inflammation	2	6	3	3	0	0	5	0	0	1	0
Untreated skin	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation	0	0	0	0	1	0	0	0	0	0	*
Liver	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Round cell infiltration	2	0	6	3	1	1	3	5	0	1	*
Kupffer cell proliferation	0	2	5	3	1	0	0	0	0	0	0
Necrosis	0	1	2	1	0	0	0	1	0	0	0
Kidneys	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Hyaline droplets	7	6	10	10	10	0	0	0	0	0	0
Mineralization	0	0	0	0	0	8	10	8	9	10	9
Tubular atrophy	0	1	0	2	0	0	0	0	0	0	0
Spleen	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Increased erythropoiesis	9	10	8	10	8	9	9	9	10	9	*
Recovery Sacrifice											
Treated skin	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Hyperkeratosis	0	0	0	0	0	0	*	0	0	*	*
Liver	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Round cell infiltration	1	0	4	2	1	0	2	3	1	1	0
Kupffer cell proliferation	0	0	1	2	0	0	0	0	0	0	0
Kidneys	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Hyaline droplets	3	5	5	5	5	0	0	0	0	0	0
Mineralization	1	0	0	0	0	4	*	5	5	5	5
Tubular atrophy	1	0	0	1	0	0	0	0	0	0	0
Spleen	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Increased erythropoiesis	3	1	4	1	3	5	1	2	4	3	3

[a]Numbers in parentheses represent number of animals from which tissue was examined.

D. STUDY AUTHORS' CONCLUSIONS:

Endosulfan was administered dermally to Wistar rats for 4 weeks at doses of 27 54, or 81 mg/kg for males and 9, 12, 18, or 36 mg/kg for females. Additional groups of animals were dosed with the formulation base administered in the vehicle. A recovery period of 4 weeks followed dosing. Five females died during the study. Transient clinical signs were exhibited, which were indicative of central nervous system involvement. Endosulfan elicited marginal to slight dermal irritation, while the incidence and severity of irritation elicited from the formulation base was minimal to moderate. Microscopic findings of hyperkeratosis and inflammation in endosulfan-dosed and control animals were considered to reflect mechanical irritation resulting from hair clipping. The severity and incidence of these findings were more prominent in rats treated with the formulation base; this was considered to reflect a slight irritating effect. Slight changes in clinical chemistry levels (increased ASAT and ALP, decreased albumin and albumin/globulin ratio) were considered to be indicative of faint impairment of hepatic function. Plasma cholinesterase activity was decreased in females dosed with endosulfan at 12, 18, or 36 mg/kg. The NOEL for systemic toxicity was 54 mg/kg in males and 9 mg/kg in females.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were adequate. However, the study report contains a number of deficiencies. There is some discrepancy as to the guidelines that were followed for the conduct of the study. Page 1 of the study report references Guideline No. 82-3 (90-Day Subchronic Toxicity Study) of the EPA Pesticide Assessment Guidelines, 1982, and page 10 references Guideline No. 82-2 (21-Day Repeated Dose Dermal Toxicity Study). The study was conducted in accordance with Guideline No. 82-2.

The group mean dermal irritation scores presented in the study report were calculated based on individual daily erythema and edema scores for males and females combined. It is not appropriate to combine the male and female data from any one test group when calculating group mean data, especially since the dose levels selected for the endosulfan-treated groups were different for males and females. Dose levels were based on previous studies conducted with the test material, which indicated that female rats were more sensitive than males. Following review of these data, the reviewers concluded that recalculation of the group mean values to obtain separate mean values for males and females from each test group would not change the overall interpretation of the study results. However, this issue should be addressed and clarified within the text of the study report.

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Incidence data for the treatment-related signs of systemic toxicity noted postdosing (i.e., tremors, trismus, Straub-tail, spasms) were not presented within the study report. Although the study authors state that these observations are part of the raw data, it is not possible to adequately assess the reported results without the inclusion of individual and/or summary data for these signs.

The reviewers noted that the quality assurance statement was dated 3 days after the report issue date. Homogeneity and concentration analyses for one sample (81 mg/kg) dose level were unacceptable (mean concentration of 68.3% of nominal, homogeneity within ±70%); the study author stated that these unacceptable results were due to an error during sample analysis and not to sample preparation. This explanation is unsatisfactory without additional data. The study authors did not report that study controls were dosed dermally in the same manner as those treated with endosulfan; this should be clarified.

The study author considered the body weight loss of dosed animals to be incidental. Based on the consistency of the weight loss (even though slight), the reviewers consider this finding, in males, to be a compound-related effect. The body weight loss in females may not be a result of dosing, since the pretest weight of females dosed with 18 mg/kg was slightly reduced (3.4%) when compared with that of female controls; many of the animals with low initial body weights retained low weights; and females dosed with 36 mg/kg did not exhibit reduced body weight.

Results of clinical biochemistry would have been easier to interpret with appropriate historical ranges of laboratory control Wistar rats. This was of importance when considering the significance of changes in ALP, albumin, and total protein. The reviewers do not agree with the authors' interpretation that the slight decreases in albumin and total protein following recovery were a result of impairment of hepatic function, since no hepatic effects were found in organ weights or histopathology. Plasma cholinesterase activities of females dosed with 12, 18, and 36 mg/kg were depressed (78, 71, and 68% of concurrent control activity, respectively) following dosing. Slight changes (less than 20% from levels of concurrent controls) in RCHE and BCHE activities were considered to be the result of normal biological variation. The reviewers do not consider these cholinesterase effects to be of any toxicological importance. Appropriate historical ranges of cholinesterase activity in laboratory control Wistar rats are necessary to confirm this conclusion.

Based on mortality in females and clinical signs indicative of central nervous system involvement in both males and females, the LOEL for systemic toxicity is 81 mg/kg in males and 12 mg/kg in females, and the NOEL is 54 mg/kg in males and 9 mg/kg in females. The formulation base was more toxic dermally than treatment with endosulfan.

APPENDIX A

Modified Draize Scoring System

A 39279

PROJECT 094590
SULFAN EMULSIFIABLE CONCENTRATE

INICAL SIGNS AT THE APPLICATION SITE

lanatory notes for summary table

ring for individual records

thema	: No erythema	0
	Slight erythema (barely perceptible)	1
	Well- defined erythema	2
	Severe erythema (beet redness) to slight eschar formation (injuries in depth)	3 4
ema	: No edema	0
	Slight edema (barely perceptible)	1
	Well-defined edema (area well-defined by definite raising)	2
	Moderate edema (raised approx. 1mm)	3
	Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4
ssures and cabs	: Minimal	1
	Moderate	2
	Severe	3

alculation of dosage group mean values :

$$\frac{\sum(\text{individual scores})}{\text{number of animals per group}}$$

laximal mean score :

Erythema, edema : 4
Fissures, scabs : 3

APPENDIX B

Summary Table of Dermal Clinical Signs

Appendix B*

RCC PROJECT 094590
ENDOSULFAN EMULSIFIABLE CONCENTRATE

CLINICAL SIGNS, LOCAL - SUMMARY TABLE

WEEK	GROUP	ERYTHEMA	EDEMA	FISSURES	SCABS
1	1	.0	.0	.0	.0
1	2	.1	.0	.0	.0
1	3	.0	.0	.0	.0
1	4	.0	.0	.0	.0
1	5	.0	.0	.0	.0
1	6	.0	.0	.0	.0
2	1	.0	.0	.0	.0
2	2	.8	.0	.0	.0
2	3	.0	.0	.0	.0
2	4	.1	.0	.0	.0
2	5	.0	.0	.0	.0
2	6	.0	.0	.0	.0
3	1	.0	.0	.0	.0
3	2	1.0	.2	.0	.0
3	3	.0	.0	.0	.0
3	4	.2	.1	.0	.0
3	5	.3	.0	.0	.0
3	6	.3	.0	.0	.0
4	1	.0	.0	.0	.0
4	2	1.1	.5	.0	.0
4	3	.0	.0	.0	.0
4	4	.1	.1	.0	.0
4	5	.1	.0	.0	.0
4	6	.1	.0	.0	.0
5	1	.0	.0	.0	.0
5	2	1.1	.4	.0	.0
5	3	.0	.0	.0	.0
5	4	.1	.0	.0	.0
5	5	.1	.0	.0	.0
5	6	.1	.0	.0	.0
6	1	.0	.0	.0	.0
6	2	.4	.0	.0	.0
6	3	.0	.0	.0	.0
6	4	.0	.0	.0	.0
6	5	.0	.0	.0	.0
6	6	.0	.0	.0	.0
7	1	.0	.0	.0	.0
7	2	.3	.0	.0	.0
7	3	.0	.0	.0	.0
7	4	.0	.0	.0	.0
7	5	.0	.0	.0	.0
7	6	.0	.0	.0	.0
8	1	.0	.0	.0	.0
8	2	.1	.0	.0	.0
8	3	.0	.0	.0	.0
8	4	.0	.0	.0	.0
8	5	.0	.0	.0	.0
8	6	.0	.0	.0	.0
9	1	.0	.0	.0	.0
9	2	.1	.0	.0	.0
9	3	.0	.0	.0	.0
9	4	.0	.0	.0	.0
9	5	.0	.0	.0	.0
9	6	.0	.0	.0	.0

54 records selected.

* Table excerpted from the report (RCC Project No. 094590)

007937

EPA No.: 68D80056
DYNAMAC No.: 210-B
TASK No.: 2-10B
January 16, 1990

DATA EVALUATION RECORD

ENDOSULFAN

Subchronic Dermal Toxicity Study in Rats

PPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 11/16/90

007937

EPA No.: 68D80056
DYNAMAC No.: 210-B
TASK No.: 2-10B
January 16, 1990

DATA EVALUATION RECORD

ENDOSULFAN

Subchronic Dermal Toxicity Study in Rats

VIEWED BY:

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Principal Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: January 16, 1990

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007937

DATA EVALUATION RECORD

GUIDELINE § 82-2

UDY TYPE: Subchronic Dermal Toxicity Study in Rats.

ID NUMBER: 410485-06.

ST MATERIAL: Endosulfan.

ONYM(S): HOE 002 671; thiodan; benzoepin; 6,7,8,9,10,10-hexa-loro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathi in-3-oxide.

DY NUMBER(S): 87.0664.

NSOR: Hoechst Celanese Corporation, Somerville, NJ.

TING FACILITY: Pharma Research Toxicology and Pathology, chst AG, Federal Republic of Germany.

LE OF REPORT: Endosulfan-Water-Dispersible Powder (50%) (Code: 002671 OI WP50 A501) Subchronic Dermal Toxicity (21 Treatments 30 Days) in the Wistar Rat.

HOR(S): E. Ebert.

ORT ISSUED: May 17, 1987.

CONCLUSIONS

Groups of 11 male and 11 female Wistar rats were dosed with 21 dermal applications of endosulfan over a period of 30 days at dose levels of 0, 160, or 640 mg/kg/day (males) or 0, 80, or 160 mg/kg/day (females). Six animals/sex received doses of 40 mg/kg/day for the same duration. Following the dosing period, groups of five animals/sex/dose (control, mid-, and high-dose groups) were maintained without treatment for a 25-day recovery period. One mid- and three high-dose females died during the study as a result of cardiovascular insufficiency; one mid-dose female exhibited clinical intoxication. There were no signs of neurological disturbance. Body weights and body weight gains of high-dose males were depressed from study day 10 to study termination; body weights continued to be depressed during the recovery period. Body weights of dosed females were not affected. Slight dermal irritation (erythema, dryness, scaling) of mid- and high-dose males and females receded by the end of the recovery period. There were no macroscopic or microscopic dermal changes. Reticulocyte counts of mid- and high-dose males and high-dose females and levels of cholesterol and total lipids in high-dose females were increased following dosing. Serum cholinesterase activity of mid- and high-dose females was depressed following dosing and remained depressed following the recovery period. No effects were exhibited on erythrocyte or brain cholinesterase. There were no changes in organ weights or systemic pathology that were considered to be a result of dosing. Based on body weight loss in males and mortality in females, the systemic LOEL is 640 mg/kg in males and 80 mg/kg in females, and the NOEL is 160 mg/kg in males and 40 mg/kg in females.

Classification: CORE Minimum (see Reviewers' Discussion and Interpretation of Results).

A. MATERIALS:

1. Test Compound: Endosulfan (50%); description: beige powder; batch No. PC86-83; purity: 49.5% active ingredient.

2. Test Animals: Species: rat; strain: Wistar [Hoe:Wiskf(SPF71)]; age: males--9 weeks; females--11 weeks at study initiation; weight: males--236 to 267 g; females--195 to 240 g at study initiation; source: Hoechst AG, Kastengrund, SPF breeding colony.

B. STUDY DESIGN:

1. Animal Assignment: Animals were assigned randomly to the following test groups and were then acclimated (unspecified duration) to laboratory conditions prior to study initiation:

Test Group	Dermal Dose (mg/kg) Males	Females	Concentration% (w/v) Males	Females	Main Study[a] (30 Days) Males	Females	Recovery[b] (25 Days) Males	Females
1 Control[c]	0	0	0	0	6	6	5	5
2 Low (LDT)	40	40	2	2	6	6	-[d]	-
3 Mid (MDT)	160	80	8	4	6	6	5	5
4 High (HDT)	640	160	32	8	6	6	5	5

[a]Main animals were sacrificed 1 day following final dermal application.

[b]Recovery animals were sacrificed 25 days (males) or 24 days (females) following the final dermal application.

[c]Control animals received 2 mL/kg physiological saline.

[d]No recovery observation for low-dose animals.

The dose levels selected for male rats for this study were based on a 5-day prestudy in which aqueous dispersions [1.6 to 50% (w/v); 32 to 1000 mg/kg] of endosulfan were applied dermally to groups of three males and three females. Dermal irritation was observed after two to three applications of 37.5 and 50% (w/v) endosulfan. A 32% preparation of endosulfan was considered to be the highest test concentration that would not produce severe dermal lesions during 21 applications over a period of 30 days, and was selected for the subchronic study. Because of technical restrictions, the maximum application volume was determined to be 2 mL/kg, and 640 mg/kg was selected as the highest possible test concentration. Dose levels selected for female rats were based on a previous subchronic dermal toxicity study (report Nos. 84.0223 and 84.0321). No other information regarding this study was reported.

Rats were housed individually in a room with temperature and humidity controls set at 19-22°C and 35-60%, respectively, with a 12-hour light/dark cycle.

2. Dose Preparation: Dispersions of endosulfan in deionized water at a constant application volume of 2 mL/kg at the required concentrations were prepared daily. Concentration, stability, and homogeneity analyses were performed on each test preparation. Testing of 2, 4, 8, and 32% dispersions of the test material showed the material to be stable for up to 5 hours. Concentration and homogeneity data were not reported.

3. Preparation of Application Site: Prior to dosing and weekly thereafter, the hair was removed from the dorsal treatment sites (approximately 10% of the total body surface) by clipping. The test material in a volume of 2 mL/kg was applied to the clipped area, 5 days/week, for a total of 21 applications in 30 days. The area was covered and wrapped with an occlusive bandage for 6 hours; after this time, the test site was washed with warm water. Control animals were treated with physiological saline in volumes equal to test animals. At the end of the dosing period, six animals/sex/dose were sacrificed. Recovery animals (five animals/sex/dose for control, mid-, and high-dose groups) were sacrificed following an additional 25 days.

4. Food and Water Consumption: Animals received Altromin 1324 pelleted rat diet and water ad libitum.

5. Statistics: The following procedures were utilized in analyzing the numerical data: body weights, water consumption, hematology (with exception of differential blood count and methemoglobin) and clinical chemistry parameters, urinalyses, and organ weights were analyzed using the parametric methods of Dunnett and Sidak, the nonparametric methods of Nemenyi/Sidak and Nemenyi/Dunnett, the T-test, and the Wilcoxon test when appropriate.

6. Quality Assurance: A quality assurance statement was signed and dated March 10, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily (once daily on weekends and holidays) for signs of morbidity and mortality. The animals were examined weekly for neurological disturbances, opacity of the refracting media of the eyes, damage to the oral mucosa, and impairment of dental growth. During the acclimation period and 2 to 3 days following final endosulfan dermal application, the recovery animals were examined for spontaneous neurological behavior, behavioral reaction to various manipulations, reflexes, and autonomous clinical signs. Based on the grading system of Draize, the visible changes and irritant effects of the test material on the treated skin were examined prior to each application.

   Results: No deaths or compound-related clinical signs were seen in dosed males or low-dose females. One mid-dose female (animal No. 60) died on study day 21 without showing previous clinical effects; one mid-dose female (animal No. 61) exhibited dacryohemorrhea and a blood-encrusted snout

on study day 22; this animal remained alive until scheduled sacrifice. Three high-dose females died (animal No. 78 on day 3, animal No. 71 on day 11, and animal No. 77 on day 24) without showing previous clinical effects. These animals, as well as the one mid-dose female, were reported to have died as a result of cardiovascular insufficiency. There were no signs of neurological disturbance, changes in the eyes, damage to the oral mucosa, or impairment of dental growth.

Slight dermal erythema, dryness, and scaling were exhibited in mid- and high-dose males and females beginning on study days 6 to 7; all dermal effects receded by study day 50 in males and day 36 in females (Tables 1 and 2). The dermal erythema never exceeded a grade of 1, which according to Draize, is barely perceptible. Low-dose males and females exhibited dryness and scaling of the skin to a lesser extent when compared to other dosed animals.

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

Results: Representative data on mean body weights and mean weight gains are summarized in Tables 3 and 4, respectively. Body weights of males were recorded within 1 day of those of females. Mean body weights and body weight gains of high-dose males were depressed from study day 7 to study termination (4-10%). These decreased body weights were statistically significant ($p < 0.05$) from study day 10 to study termination. Mean body weights of these animals did not recover following dosing; body weight gains of high-dose males (+47 g) were also slightly depressed when compared to those of concurrent controls (+55 g) during the recovery period (weeks 30 to 53). Since no animal deaths occurred between study weeks 30 and 53, differences in body weight gain were calculated by subtracting respective body weight gains (Table 4). Mean body weights and body weight gains of mid-dose males were slightly but not significantly decreased during this same time. Body weights and body weight gains of low-dose males and dosed females were similar to those of concurrent controls.

On study day 24, body weights and body weight gains of control and dosed females were depressed by approximately 10% from the body weights reported on day 22 (previous weighing); body weights had recovered by the following weighing on day 30. This finding was not addressed by the study author. The pattern of body weight gains in females was found to be inconsistent over time when comparing differences between weeks 0-7, 8-15, 16-25, and 26-30 (Table 4).

TABLE 1. Summary of Dermal Observations[a] in Male Rats Administered Endosulfan for 30 Days Followed by a 25-Day Recovery

Dose Group (mg/kg/day)	Observation	Test Day 2	8	14	21	28	35	42	52
0[b]	Erythema	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0
	Dryness	0	0	0	0	0	0	0	0
	Scabbing	0	0	0	0	0	0	0	0
	Eschar[d]	0	0	0	0	0	0	0	0
	Scaling	0	0	0	0	0	0	0	0
40[c]	Erythema	0	0	0	0	0			
	Edema	0	0	0	0	0			
	Dryness	0	2	1	4	3			
	Scabbing	0	0	0	1	1			
	Eschar	0	0	0	0	0			
	Scaling	0	0	0	4	1			
160[b]	Erythema	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0
	Dryness	0	9	11	10	10	3	0	0
	Scabbing	0	0	1	1	0	0	0	0
	Eschar	0	0	1	5	4	0	0	0
	Scaling	0	7	9	6	7	2	0	0
640[b]	Erythema	0	0	1	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0
	Dryness	0	11	11	11	10	5	3	0
	Scabbing	0	0	3	0	1	0	0	0
	Eschar	0	0	7	9	7	3	2	0
	Scaling	0	11	11	11	10	4	3	0
	Skin discoloration	0	0	0	6	0	0	0	0

[a]Number presented represents the number of rats in each group exhibiting a given observation, based on eleven animals/sex/dose at study initiation.

[b]Six of eleven animals sacrificed on study day 30.

[c]Six of six animals sacrificed on study day 30.

[d]Large- or small-scale separation from skin is regarded as eschar in this study report.

TABLE 2. Summary of Dermal Observations[a] in Female Rats Administered Endosulfan for 30 Days Followed by a 25-Day Recovery

Dose Group (mg/kg/day)	Observation	Test Day 2	8	14	21	28	35	42
0[b]	Erythema	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0
	Dryness	0	0	0	0	0	0	0
	Scabbing	0	0	0	0	0	0	0
	Eschar[d]	0	0	0	0	0	0	0
	Scaling	0	0	0	0	0	0	0
40[c]	Erythema	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0
	Dryness	0	0	3	2	2	0	0
	Scabbing	0	0	0	0	0	0	0
	Eschar	0	0	0	0	0	0	0
	Scaling	0	0	3	0	0	0	0
80[b]	Erythema	0	1	0	0	0	0	0
	Edema	0	0	0	0	0	0	0
	Dryness	0	2	8	6	2	0	0
	Scabbing	0	0	0	0	0	0	0
	Eschar	0	0	0	0	0	0	0
	Scaling	0	2	8	1	2	0	0
160[b]	Erythema	0	4	3	0	0	0	0
	Edema	0	0	0	0	0	0	0
	Dryness	0	8	9	7	4	2	0
	Scabbing	0	0	0	0	0	0	0
	Eschar	0	0	0	0	0	0	0
	Scaling	0	6	9	3	3	1	0

[a]Number presented represents the number of rats in each group exhibiting a given observation; based on eleven animals/sex/dose at study initiation with the exception of the low dose (40 mg/kg) with six animals/sex. Deaths of mid- and high-dose animals occurred on days 3, 11, 21, and 24.

[b]Six of eleven animals sacrificed on study day 30.

[c]Six of six animals sacrificed on study day 30.

[d]Large- or small-scale separation from skin is regarded as eschar in this study report.

TABLE 3. Representative Results of Mean Body Weights (± S.D.) of Rats Administered Endosulfan Dermally for 30 Days[a]

Dose Group	Mean Body Weights (g) at Day 1	7	15	25	30	32	43	53
				Males				
0[b]	286 ± 11	301 ± 13	319 ± 18	324 ± 23	345 ± 23	345 ± 18	381 ± 22	404 ± 17
40[c]	288 ± 12	302 ± 12	318 ± 14	315 ± 9	338 ± 10			
160	282 ± 12	295 ± 14	309 ± 17	311 ± 18	328 ± 19	326 ± 13	359 ± 16	377 ± 16*
640	281 ± 11	288 ± 13	301 ± 11*	298 ± 11*	317 ± 12*	313 ± 7*	344 ± 11*	365 ± 17*
	Days 1	8	17	24	30	31	42	52
				Females				
0[b]	237 ± 10	237 ± 11	238 ± 14	219 ± 12	241 ± 13	243 ± 17	251 ± 17	261 ± 60
40[c]	232 ± 12	235 ± 8	242 ± 8	222 ± 11	250 ± 11			
80	239 ± 12	231 ± 14	240 ± 18	220 ± 14	247 ± 16	255 ± 12	267 ± 19	277 ± 14
160[d]	236 ± 8	231 ± 10	240 ± 7	216 ± 9	246 ± 15	259 ± 18	260 ± 20	264 ± 19

[a]Based on eleven animals/sex/dose at study initiation with the exception of the low dose with six animals/sex.

[b]Six of eleven control, mid-, and high-dose animals sacrificed on study day 30.

[c]Six of six low-dose animals sacrificed on study day 30.

[d]No statistics conducted on high-dose females, since group contained less than four animals.

*Significantly different from control values ($p < 0.05$).

TABLE 4. Representative Results of Mean Body Weight Gains (± S.D.) of Rats Administered Endosulfan Dermally for 30 Days[a,b]

Dose Group (mg/kg/day)	Mean Body Weight Gain (g/rat) Between Days						
	0-7	0-15	0-25	0-30	0-32	0-43	0-53
				Males			
0[c]	53 ± 11	71 ± 15	76 ± 19	97 ± 19	93 ± 13	130 ± 15	152 ± 9
40[d]	51 ± 7	67 ± 8	64 ± 12	87 ± 7			
160	47 ± 9	62 ± 13	63 ± 15	80 ± 15	80 ± 13	113 ± 16	131 ± 16
640	39 ± 11	52 ± 10	49 ± 11	68 ± 13	63 ± 12	94 ± 14	115 ± 20
	Days						
	0-8	0-17	0-24	0-30	0-31	0-42	0-52
				Females			
0[c]	10 ± 5	11 ± 7	-8 ± 6	15 ± 7	15 ± 8	23 ± 9	33 ± 13
40[d]	9 ± 4	16 ± 5	-5 ± 8	24 ± 7			
80	4 ± 17	13 ± 19	-7 ± 19	21 ± 21	32 ± 26	43 ± 34	53 ± 27
160	3 ± 8	12 ± 6	-12 ± 8	19 ± 13	33 ± 15	33 ± 16	38 ± 16

[a]Based on eleven animals/sex/dose at study initiation with the exception of the low dose with six animals/sex; weight gains from weeks 32 to 53 were based on five rats/sex in the control, mid-, and high-dose groups.

[b]Statistical analyses were not performed for these data.

[c]Six of eleven control, mid-dose, and high-dose animals sacrificed on study day 30.

[d]Six of six low-dose animals sacrificed on study day 30.

3. Food Consumption and Compound Intake: Consumption was determined and mean daily dietary consumption was calculated at the same intervals as body weights. Water consumption was determined weekly for a 16-hour interval.

Results: Food consumption of dosed males and females was found to be similar to that of concurrent controls throughout the study. In correlation with the body weight reduction in control and dosed females on study day 24, food consumption at this time decreased markedly by 42-49% from that reported on day 22; food consumption of control and dosed males on study day 25 decreased by 23-34% from that reported on day 22. Food consumption levels recovered by the following weighing on day 30. This finding was not addressed by the study author.

Mean total food consumption over the study period was 9.0, 9.1, 9.0, and 9.1 g/100 g body weight/day for control, low-, mid-, and high-dose males, and 8.8, 8.9, 8.7, and 8.5 g/100 g body weight/day for control, low-, mid-, and high-dose females, respectively. There were no compound-related effects on water consumption.

4. Ophthalmology: Ocular examinations were performed during the acclimatization phase and for 2 to 3 days following the final endosulfan dermal application on the recovery animals. Examinations included width of palpebral fissures, conjunctivae, crystalline lens, iris, and anterior eye chamber.

Results: There were no compound-related effects on ophthalmology.

5. Hematology and Clinical Chemistry: Blood was collected from the retrobulbar venous plexus for hematology and from the vena cava cranialis for clinical analyses from six animals/sex/dose at termination of dosing and all remaining animals at termination of recovery. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)†
X Hemoglobin (HGB)†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
X Platelet count†
X Reticulocyte count (RETIC)
Red cell morphology
X Leukocyte differential count†
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Coagulation: thromboplastin time (PT)†
X Activated partial thromboplastin time (APTT)
X Heinz bodies

†Recommended by Subdivision F (October 1982) Guidelines for subchronic dermal toxicity studies.

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Results: Reticulocyte counts of mid- and high-dose males and high-dose females were slightly but significantly ($p < 0.05$) increased following dosing. This effect was dose related in males; however, there was no correlating decrease in erythrocyte counts (RBC), hemoglobin concentration (HGB), or hematocrit (HCT). This finding was not considered to be compound related by the study authors. Other sporadic changes (coagulation time in low-dose males following dosing, platelet counts in mid-dose females following recovery) were slight, and found in only one sex and at only one dose level.

b. Clinical Chemistry

	Electrolytes
X	Calcium†
X	Chloride†
	Magnesium
X	Phosphorus†
X	Potassium†
X	Sodium†

	Enzymes
X	Alkaline phosphatase (ALP)
X	Cholinesterase (serum, erythrocyte, brain)
	Creatinine phosphokinase
X	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (SGPT)†
X	Serum aspartate aminotransferase (SGOT)†
X	Gamma glutamyltransferase (GGT)

	Other
	Albumin†
	Albumin/globulin ratio
X	Blood creatinine†
X	Blood urea nitrogen†
X	Cholesterol
	Globulins
X	Glucose
X	Total bilirubin†
	Direct bilirubin
X	Total protein†
X	Triglycerides
X	Uric acid
X	Total lipids
X	Electrophoresis

Results: Cholinesterase activity data are presented in Table 5. Following dosing, serum cholinesterase (SCHE) activities of mid- and high-dose females were significantly ($p < 0.05$) depressed in a dose-related manner (72 and 54% of concurrent control activity, respectively); SCHE activities of these animals remained depressed following recovery. The study authors reported this depression of SCHE in females to be a result of hepatic impairment of cholinesterase or pseudocholinesterase biosynthesis. SCHE activities of low- and high-dose males were slightly but significantly ($p < 0.05$) depressed (87% of concurrent control activity) following dosing; this depression following dosing was not dose related and was not found following recovery.

†Recommended by Subdivision F (October 1982) Guidelines for subchronic dermal toxicity studies.

TABLE 5. Mean Cholinesterase Activity in Rats Administered Endosulfan Dermally for 30 Days Followed by a 25-Day Recovery[a]

Dose Group mg/kg/day	Cholinesterase Activity[b] SCHE (U/L) Post-dosing	SCHE (U/L) Post-recovery	RCHE (U/L) Post-dosing	RCHE (U/L) Post-recovery	BCHE (U/kg) Post-dosing	BCHE (U/kg) Post-recovery
			Males			
0	467 ± 39	328 ± 38	332 ± 36	442 ± 37	4267 ± 147	4152 ± 227
40[c]	407 ± 31*(87)[d]		424 ± 163(127)		4538 ± 455(106)	
160	426 ± 45(91)	332 ± 69(101)	356 ± 29(107)	456 ± 24(103)	4579 ± 264(107)	4029 ± 253(97)
640	405 ± 30*(87)	299 ± 59(91)	379 ± 22(114)	469 ± 26(106)	4891 ± 263(115)	3847 ± 246*(93)
			Females			
0	1318±246	1031 ± 125	514 ± 90	514 ± 53	4173 ± 175	3853 ± 167
40	1005±248(76)		507 ± 50(99)		4257 ± 176(102)	
80	952±121*(72)	782 ± 124*(76)	486 ± 107(95)	495 ± 36(96)	3944 ± 409(95)	3615 ± 572(94)
160	709±79*(54)	798 ± 175[e](77)	548 ± 71(107)	477 ± 20(93)	4074 ± 266(98)	3712 ± 194(96)

[a]Cholinesterase activity of males was based on six animals/dose postdosing and five animals/dose postrecovery; cholinesterase activity of females was based on six animals/dose for control and low-dose groups and five animals/dose for mid- and high-dose groups postdosing, and on five animals/dose for control and mid-dose groups and three animals for high-dose group postrecovery.

[b]Abbreviations: SCHE = serum cholinesterase; RCHE = erythrocyte cholinesterase; BCHE = brain cholinesterase.

[c]Six of six low-dose animals sacrificed following dosing.

[d]Numbers in parentheses represent percentage of control.

[e]Statistics not conducted because animal number was less than 4.

*Significantly different from controls ($p < 0.05$).

Erythrocyte (RCHE) and brain (BCHE) cholinesterase activities varied slightly following dosing and recovery when compared to concurrent controls. This was considered to be a result of normal biological variation.

Cholesterol and total lipids of high-dose females were found to be significantly ($p < 0.05$) increased following dosing when compared to concurrent controls (Table 6); however, the cholesterol level of control females appears to be slightly depressed when compared to that of control males and postrecovery values. These increases in cholesterol and total lipids were not found in males, in other dosed females, or following recovery.

6. Urinalysis: Urine was collected from fasted animals at study day 24 for animals of the main groups and study day 45 for recovery animals. The CHECKED (X) parameters were examined:

X	Appearance†	X	Glucose†
X	Color	X	Ketones†
X	Volume†	X	Bilirubin†
	Specific gravity†	X	Blood†
X	pH		Nitrate
X	Sediment (microscopic)[a†]		Urobilinogen
X	Protein†		

[a]The urine of high-dose rats was examined only for sediment.

Results: There were no compound-related changes in urinalyses.

†Recommended by Subdivision F (October 1982) Guidelines for subchronic dermal toxicity.

TABLE 6. Selected Mean Clinical Chemistry Parameters (± S.D.) of Rats Administered Endosulfan Dermally for 30 Days Followed by a 25-Day Recovery[a]

Dose Group (mg/kg/day)	Cholesterol (mmol/L)		Total Lipids (g/L)	
	Post-dosing	Post-recovery	Post-dosing	Post-recovery
		Males		
0	1.82 ± 0.28	1.90 ± 0.34	3.62 ± 0.57	4.48 ± 0.59
40	1.61 ± 0.22	[b]	4.26 ± 0.33	[b]
160	1.78 ± 0.34	1.70 ± 0.13	3.51 ± 0.48	4.18 ± 0.23
640	1.89 ± 0.21	1.64 ± 0.13	3.68 ± 0.42	3.99 ± 0.30
		Females		
0	0.82 ± 0.11	1.62 ± 0.47	3.02 ± 0.42	4.76 ± 0.66
40	0.85 ± 0.11	[b]	3.20 ± 0.25	[b]
80	0.90 ± 0.20	1.48 ± 0.13	3.20 ± 0.61	4.59 ± 0.59
100	1.25 ± 0.24*	1.48 ± 0.24	4.12 ± 0.49*	4.19 ± 0.50

[a] Based on six males/dose postdosing and five males/dose postrecovery; six females/dose for control and low-dose groups and five females/dose for mid- and high-dose groups postdosing, and on five females/dose for control and mid-dose groups and three females for high-dose group postrecovery.

[b] Six of six low-dose animals sacrificed following dosing.

*Significantly different from controls ($p < 0.05$).

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta†	XX	Brain†
	Salivary glands†	XX	Heart†	X	Peripheral nerve (sciatic nerve)†
	Esophagus†	X	Bone marrow		
X	Stomach†		Lymph nodes†	X	Spinal cord (3 levels)
X	Duodenum†	XX	Spleen†		
X	Jejunum†	XX	Thymus†	XX	Pituitary†
X	Ileum†			X	Eyes (optic nerve)
X	Cecum†				
X	Colon†				
X	Rectum		Urogenital		Glandular
XX	Liver†	XX	Kidneys†	XX	Adrenals†
	Gallbladder†	X	Urinary bladder†		Lacrimal gland
X	Pancreas†	XX	Testes†		Mammary gland
		X	Epididymides		Parathyroids†
		X	Prostate	XX	Thyroids†
		X	Seminal vesicle		Harderian glands
	Respiratory	XX	Ovaries		
	Trachea	X	Uterus†		
XX	Lung†				
					Other
					Bone (sternum)
					Skeletal muscle
				X	Skin (treated, untreated)†
				X	All gross lesions and masses†

A complete inventory of tissues was examined for all rats in the control and high-dose groups. Heart, kidneys, liver, spleen, lungs, and skin (treated, untreated) were examined for all low- and mid-dose animals.

Results:

a. Organ Weights: There were no compound-related changes in organ weights. Following dosing, absolute kidney weights of mid-dose females were reported to be significantly ($p < 0.05$) increased when compared to those of concurrent controls; however, this increase is due to the inflated value of one outlier (animal No. 57) noted by the study authors to be incorrectly tabulated as a result of technical error. When this mean is

†Recommended by Subdivision F (October 1982) Guidelines for subchronic dermal toxicity.

calculated without the inflated individual value, it is found to be similar to the mean kidney weights of concurrent controls and other dosed females. Relative kidney weights of mid- and high-dose males were found to be significantly ($p < 0.05$) increased; the study author considered these increases to be the result of reduced body weights and to be unrelated to dosing.

Following recovery, slight changes in organ weights of mid- and/or high-dose males were considered to be incidental; changes were slight and sporadic and had not occurred during dosing.

b. Gross Pathology: There were no gross pathological changes that were considered to be related to dosing. Sporadic findings (e.g., epididymal swelling, blood clots of the gastric mucosa) were considered to be incidental. No dermal changes were observed.

c. Microscopic Pathology: There were no histological changes that were considered to be a result of dosing dermally with endosulfan. The females that died during the study exhibited slight to moderate edema and congestion of the heart as a result of cardiovascular insufficiency. Sporadic findings were considered to be incidental and were within the incidence range of concurrent controls. No microscopic dermal changes were observed.

D. STUDY AUTHORS' CONCLUSIONS:

Dermal administration of endosulfan at doses of 40, 160, or 640 mg/kg/day in male Wistar rats and 40, 80, or 160 mg/kg in female Wistar rats for 30 days caused mortality at the mid and high dose in females, dacryohemorrhea, and blood-encrusted snout in one mid-dose female, and reduction of body weight gains in high-dose males. Endosulfan caused slight dermal changes that receded by study week 2. The NOEL is 40 mg/kg in females and 160 mg/kg in males.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were adequate. However, the study report contains some discrepancy as to the guidelines that were followed for the conduct of the study. Page 1 of the study report references Guideline No. 82-3 (90-Day subchronic toxicity study) of the EPA Pesticide Assessment Guidelines, 1982, and page 9 of the study report references Guideline No. 82-2 (21-day Repeated Dose Dermal toxicity study). The reviewers assume that the study was conducted in

accordance with Guideline No. 82-2. Although this study does not qualify for Guideline No. 82-3, it does fulfill guidance requirements for No. 82-2 with the exception of the measurement of serum albumin levels included among the parameters tested for clinical chemistry.

Even though the 5-day preliminary study conducted prior to this 30-day study determined that a 32% preparation of endosulfan was considered to be the appropriate test concentration, the study author used a 50% test concentration for this dermal testing.

Results of clinical biochemistry would have been easier to interpret with appropriate historical ranges of laboratory control Wistar rats. For example, cholesterol values of control females appeared to be slightly depressed following dosing. This was of importance when considering the significance of comparatively increased values of dosed females.

Serum cholinesterase (SCHE) activities of mid- and high-dose females were depressed (72 and 54% of concurrent control activity, respectively) following dosing and remained depressed following the recovery period. The SCHE activities of low- and high-dose males were slightly depressed (87% of concurrent control activity) following dosing. No effects were exhibited on erythrocyte or brain cholinesterase. Consequently, the reviewers do not consider these effects on SCHE to be of any toxicological importance. Appropriate historical ranges of cholinesterase activity in laboratory control Wistar rats are necessary to confirm this conclusion. In addition, the reviewers do not agree with the authors' interpretation of impaired cholinesterase biosynthesis, since no hepatic effects were found in organ weights or histopathology.

The study author indicated that the signs of dermal irritation in treated males and females had receded by study week 2. According to tabulated data, all dermal effects had receded by study day 50 in males and day 36 in females. Dryness of the skin is regarded by the reviewers as a questionable sign of dermal irritation. Based on body weight loss in males and mortality in females, the systemic LOEL is 640 mg/kg in males and 80 mg/kg in females, and the NOEL is 160 mg/kg in males and 40 mg/kg in females.