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JUL 30 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of Subchronic Dermal Toxicity in the Rat Using Endosulfan

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

FROM: Margaret L. Jones M.L. Jones 7/28/86
Review Section III
Toxicology Branch (TS-769)

THROUGH: Marcia Van Gemert, Ph.D., Head M Van Gemert 7.29.86
Review Section III

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

Compound: Endosulfan Tox. Chem. No: 420

Registration No: 154124 Registrant: American Hoechst

Accession Nos: 257682, 257683 Project No: 488

Action Requested: Review the Subchronic Dermal Toxicity Study in the Rat submitted as follow-up to the 1982 Endosulfan Registration Standard. These data were listed as gaps in the standard.

Conclusion: Endosulfan was applied to the skin of male and female Wistar rats at doses of 0, 12, 48, 96, and 192 mg/kg in males and 0, 3, 6, 12, and 48 mg/kg in females for 21 applications over 29 days. No observed effects levels for subchronic dermal toxicity (repeated dose dermal) in the rat were established as follows:

NOEL (female rat) = 12 mg/kg
NOEL (male rat) = 96 mg/kg

There was 18% mortality observed in females at the highest dose tested (48 mg/kg), and 36% mortality in males at the highest dose tested (192 mg/kg).

Endosulfan was non-irritating at all doses tested.

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The study report states: "One female in each of the three lowest dose groups died on day 18 from no evident cause. These mortalities were attributed to the application technique employed, and for this reason this study was followed immediately by another study using a modified method of application."[1] The method of application was not sufficiently described in order to conclude whether or not the method was at fault. However, the deaths do not appear to demonstrate significant toxicity of Endosulfan or to warrant a repeat study, which the report concludes.

Classification: core Minimum

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p. 17, Hoechst AG, Report No. A30754.

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DATA EVALUATION REPORT

Study Type: Subchronic Dermal Toxicity in Rats Tox.Chem.No: 420

Accession Nos: 257682, 257683 MRID No: None

Test Material: Endosulfan Technical

Synonyms: Thiodan®, Thionex®

Study Number(s): Report No. A30754 (Translation of Doc. No: A30751); Documentation No: 721; Study No: 83.0118

Sponsor: American Hoechst Corporation, Agricultural Division

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

Title of Report: Endosulfan-active ingredient technical (Code: Hoe 002671 OI ZD97 0003) Testing for subchronic dermal toxicity (21 applications over 30 days) in SPF Wistar rats

Author(s): Ebert, Weigand, Kramer

Report Issued: March 11, 1985

Conclusions: Endosulfan was applied to the skin of male and female Wistar rats at doses of 0, 12, 48, 96, and 192 mg/kg in males and 0, 3, 6, 12, and 48 mg/kg in females for 21 applications over 29 days. No observed effects levels for subchronic dermal (repeated dose dermal) toxicity in the rat were established as follows:

NOEL (female rat) = 12 mg/kg
NOEL (male rat) = 96 mg/kg

There was 13% mortality observed in females at the highest dose tested (48 mg/kg), and a 36% mortality in males at the highest dose tested (192 mg/kg).

Endosulfan was non-irritating at the doses tested.

The study report states: "One female in each of the three lowest dose groups died on day 18 from no evident cause. These mortalities were attributed to the application technique employed, and for this reason this study was followed immediately by another study

using a modified method of application."[1] The method of application was not sufficiently described in order to conclude whether or not the method was at fault. However, the deaths do not appear to demonstrate significant toxicity of Endosulfan or to warrant a repeat study, which the report concludes.

Classification: core Minimum

A. Materials:

1. Test compound: Endosulfan-active ingredient technical, Description: brown flakes, Batch#: Hoe 002671 OI ZD97 0003, Purity: 97.2% (w/w), contaminants: not listed
2. Test animals: Species: Wistar rat, Strain: Hoe: WISKf (SPF71), Age: 8-10 weeks, Source: Hoechst AG, Pharma Forschung Toxikologie, Kastengrund breeding colony

B. Study Design:

1. Animal assignment: Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (mg/kg BW) male	female	Main Group (21 app. in 30 da.)	Recovery Group (21 app. in 30 da. + 14 da. recovery)
1 Control	0	0	6	5
2	12	3	6	5
3	48	6	6	5
4	96	12	6	5
5	192	48	6	5

2. Diet: Altromin-R 1324 Pellets (Altromin GmbH, Lage/Lippe) ad libitum, except for periods animals were in diuresis cages

3. Preparation of skin to be tested: At the start and once weekly, hair on nape skin area (10% body area) was removed with an Aesculap electric clipper. Test substance was applied with Record syringe 21 times over 30 days, 5 days/week. Exposure lasted 6 hours under occlusive bandage. Upon bandage removal, exposed area was washed with 20% aqueous solution of polyethylene glycol 400 and warm water. Controls were treated with vehicle in a similar manner. Vehicle was sesame oil DAB7 (Mainland GmbH, Frankfurt am Main). Volume was 2 ml/kg bodyweight.

[1] p. 17, Hoechst AG, Report No. A30754.

4. Statistics: Statistical evaluation was performed at the significance level p=0.05 for the following parameters: bodyweights at scheduled intervals using parametric methods by Dunnett and by Sidak, and distributed-free method by Nemenyi/Dunnett; hematology parameters- using the parametric method by Dunnett, distributed-free method by Nemenyi/Dunnett and Nemenyi/Sidak; clinical chemistry- using the parametric method by Nemenyi/Dunnett and parametric method by Dunnett; urinalysis- using the distributed-free method by Nemenyi/Sidak; absolute organ weights using parametric method by Dunnett and distributed-free method by Nemenyi/Dunnett; relative organ weights- using parametric method by Dunnett and distributed-free method by Nemenyi/Dunnett. Where the number of surviving animals was less than four no statistical analysis was performed.

5. Quality Assurance: Five inspections and five reports dated 8/18/83 through 3/25/85 were signed by S.J. Harston. Three inspections took place during the study in August, 1983, and two at dates in March, 1985, for an unspecified reason.

C. Methods and Results:

1. Observations: Animals were inspected daily for behaviour and general health, including mortality. Dermal irritation was assessed according to the Draize method at the time of inspection.

Results: Males tolerated doses up to 96 mg/kg. Females showed signs of toxicity at 12 mg/kg. Signs were piloerection, salivation and lacrimation. With increasing doses, blood from nose and eyes was observed in females. Deaths in males occurred at the high dose (192 mg/kg) on day 6(1) and day 9(1). Deaths in females occured at the high dose (48 mg/kg) between days 2 and 22(4) from tono-clonic convulsions. Deaths occurred (1/group) in each of the three lowest dose groups in females on day 18. Application technique was the apparent cause, and another study was initiated using a modified application method.

Reactions of treated skin: Males: From days 4-6 of application, dryness and desquamation of the skin were observed at all doses including controls. Dryness and desquamation were again observed from day 8 through 10, the majority of observations occurred at the high dose (192 mg/kg) with scattered observations at 96 and 48 mg/kg. Erythema and edema scores were zero through day 3. The highest erythema score was a "2" for well defined erythema, noted in one control and one animal at 96 mg/kg on day 4. Otherwise, scattered scores of "1" for very slight erythema, and very slight edema were noted throughout the test period. Females: From the start of application, through day 7, and from days 18-21 dryness and desquamation of the skin were observed at several doses and controls. Erythema and edema were observed intermittently from day 2-4. These were all scored "2" or "1".

The majority of symptoms described disappeared by the end

of the application period.

Conclusions: Under the conditions of this study, Endosulfan was not an irritant to the skin of rats.

2. Body weight: Animals were weighed at the start and two times weekly during the study.

Results: Initial weights were 176-194 g for males, and 171-187 g for females. These were 8-10 week old rats and they were held for 4-5 days acclimation before study initiation.

Bodyweight gains in dosed animals were not significantly different from controls during the period of test substance application. During recovery, bodyweight gains in low dose males were significantly different from controls, however, only five animals were held through this period, too few to demonstrate significant toxicity of the test compound.

3. Food consumption: Food consumption was measured at the same time body weights were measured.

Results: No significant differences between controls and treated groups were noted in absolute and relative food consumption.

4. Ophthalmological examinations were performed at autopsy. Findings will be reported in the section on autopsy and final macroscopic findings.

5. Blood was collected from animals in the main study groups at the end of the study. The following parameters were examined:

hemoglobin
erythrocytes
leucocytes
hematocrit
reticulocytes
Heinz bodies
differential blood count
thrombocytes
coagulation time
methemoglobin

Calculated values were: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concencration (MCHC).

Results: No significant differences between control and treated animals in hematology parameters were noted at examination following the period of test substance application.

6. Clinical chemistry: Blood samples were taken from the retrobulbar venous plexus.

The following clinical chemistry parameters were measured:

- sodium
- potassium
- inorganic phosphorus
- uric acid
- total bilirubin
- direct bilirubin
- creatinine
- serum glucose
- urea nitrogen (BUN)
- calcium
- chloride
- SGOT
- SGPT
- Alkaline phosphatase
- LDH
- erythrocyte (RBC), serum and brain cholinesterase
- total lipids
- total proteins

Total proteins were also examined at the end of the recovery period.

Results: A few scattered differences of statistical significance were noted between controls and dosed males at day 30 in sodium (group=96 mg/kg), chloride (96 mg/kg), SGPT (12 mg/kg), protein (192 mg/kg), serum cholinesterase (192 mg/kg), RBC cholinesterase (48 mg/kg), and brain cholinesterase (96 mg/kg). The effect on serum cholinesterase appeared to be dose-related. An apparently isolated difference of statistical significance was noted in females at day 30 in protein (group=96 mg/kg).

None of the clinical chemistry results demonstrates significant toxicity of the test substance.

7. Urinalysis: Urine was collected in diuresis cages housing animals individually from days 25-26 or 27-28. Food and water were removed prior to placing animals in the cages.

The following measurements were taken to evaluate the urine of control and dosed animals:

- appearance
- color
- protein
- glucose
- hemoglobin
- bilirubin
- pH value
- sediment

Results: No statistically significant differences between controls and treated groups in males or females were noted in urinalysis. The pH of male urine ranged from 6.1 to 6.3 and female urine ranged from 5.6 to 6.1. The urine was clear to

slightly cloudy and light to dark yellow in color.

8. Organ weights: Absolute and relative organ weights were measured for the following:

heart
lungs
liver
kidneys
spleen
brain
testes (without epididymides)/ ovaries
adrenals
pituitary
thyroid
seminal vesicles

Results: Statistically significant increases in relative kidney weights in males at 12, 96, and 192 mg/kg were noted at day 30. No statistically significant differences in absolute organ weights or in relative weight of other organs were otherwise noted. The reason for the effect of the test substance on relative kidney weight was attributed to slightly lower body weights in the males, according to the test report. The kidneys were examined at histopathology in order to investigate this effect.

9. Histopathology: The following organs and tissues were preserved for histopathology examination:

heart
lungs
liver
kidneys
spleen
stomach
jejunum
colon
urinary bladder
testes
epididymides
prostate
seminal vesicles
ovaries
uterus
thyroid
pancreas
adrenals
thymus
pituitary
brain
eye with optic nerve
bone marrow
treated skin areas
untreated skin areas

Results: The only organs examined were kidney and liver at selected dose levels. Examination was focused on these organs due to results of a previous study in which "slight histological changes... in liver"[1] had been noted.

In the liver, small Kupffer cell nodules were noted in the majority of males and females in several liver lobules. This finding was noted similarly in both control and dose groups and is apparently not related to the test substance.

In one high dose male "...discrete deposition of pigment in a few cells of the proximal straight tubule..."[1] was noted. This finding is of interest because similar descriptions appeared in previously reviewed studies, although in those instances the pigment deposition occurred consistently in the proximal convoluted tubules of the kidney.

1. Test Report A30754, p. 21.