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Changes in liver cells started with 9 mg/kg bodyweight and were found at all higher doses. Liver abnormalities were enlargement of parenchymal cells in peripheral sections, together with loss of cytoplasmic basophilia; isolated cell necroses and frequent mitoses. Females at 9 mg/kg bodyweight showed significantly increased absolute and relative spleen weight, as compared to controls.

Significant dermal irritation was not produced by the test compound. Dermal irritation for all groups was less than very slight (less than a score of one) at all examinations. However, it appears that dermal irritation was more persistent in females at 3 and 9 mg/kg bodyweight, as evidenced by average dermal irritation scores 2-3 times that of controls. Average scores in males showed no differences from controls at any dose. Although dermal irritation scores were zero at the end of the study, and although the pathology report describes dermal effects similarly in treated and control animals, there appears to be an increase in severity or prolongation of irritation at 3 and 9 mg/kg bodyweight in females.

Classification: core Minimum

Recommendations:

1. As stated in the 1982 Endosulfan Registration Standard, Toxicology Chapter, the albino rabbit is the preferred species in dermal testing. A subchronic dermal study using the rabbit is strongly recommended.

2. A dermal penetration study is also strongly recommended to measure how much of the compound enters via the dermal route.

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DATA EVALUATION REPORT

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

Accession Nos: 257684, 257685

MRID No: None

Test Material: Endosulfan Technical

Synonyms: Thiodan®, Thionex®

Study Number(s): Report No. A30753 (Translation of Doc. No: A30750)
Documentation No: 729; Study No: 83.0508

Sponsor: American Hoechst Corporation, Agricultural Division

Testing Facility: Pharma Forschung Toxikologie, Hoechst
Aktiengesellschaft, Postfach 80 03 20,
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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, Leist, Kramer

Report Issued: February 22, 1985

Conclusions: Endosulfan was applied to the skin of male and female Wistar rats (6/sex/dose) at doses of 0, 1, 3, 9, and 27 mg/kg bodyweight and to males only at 81 mg/kg bodyweight for 21 applications over 30 days. The no observed effects level (NOEL) for subchronic dermal toxicity was established as follows, based on increased mortality, liver abnormalities, and increased organ weights:

NOEL = 3 mg/kg bodyweight (male and female rat)

There was 83% mortality in females at 27 mg/kg bodyweight and 50% mortality in males at 81 mg/kg bodyweight (females were not tested at this dose). Although no deaths occurred in males at 27 mg/kg, at 9 mg/kg there were 2/6 deaths (33%), a significant increase over controls, which showed 0% mortality. Mortality appears to be a compound-related effect. The cause of death as described in the pathology report was a study-related toxic shock (see also Section C.9. of this review), females appearing to be more sensitive than males. Changes in liver cells started with 9 mg/kg bodyweight and were found at all higher doses. Liver abnormalities were enlargement of parenchymal cells in peripheral sections, together with loss of cytoplasmic basophilia; isolated cell necroses and frequent mitoses. Females at 9 mg/kg bodyweight showed significantly increased absolute

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and relative spleen weight, as compared to controls.

Significant dermal irritation was not produced by the test compound. Dermal irritation for all groups was less than very slight (less than a score of one) at all examinations. However, it appears that dermal irritation was more persistent in females at 3 and 9 mg/kg bodyweight, as evidenced by average dermal irritation scores 2-3 times that of controls. Average scores in males showed no difference from controls at any dose. Although dermal irritation scores were zero at the end of the study, and although the pathology report describes dermal effects similarly in treated and control animals, there appears to be an increase in severity or prolongation of irritation at 3 and 9 mg/kg bodyweight in females.

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: WISKE (SPF71), Age: 8-10 weeks, Source: Hoechst AG, Pharma Forschung Toxikologie, Kastengrund breeding colony

B. Study Design:

1. Animal assignment: Sixty-six rats were assigned randomly to the following test groups:

Test Group	Dose in diet (mg/kg BW/day)	Main Group (21 app. in 30 da.)	
		males	females
1 Control	0	6	6
2	1	6	6
3	3	6	6
4	9	6	6
5	27	6	6
6	81	6	-

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 Rekord 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.

4. Statistics: Statistical evaluation was performed at significance level $p=0.05$ for bodyweights at scheduled intervals- using parametric methods by Dunnett and 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: Four inspections and four reports dated 10/3/83 through 3/18/85 were signed by S.J. Harston. Two inspections took place during the study in October, 1983, one in May, 1984, and another in March, 1985.

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 before each application of vehicle or test substance.

Results: Females: Three females at 0, 3, and 9 mg/kg showed autoaggressive signs, biting at the bandage in the ventral region. No other visible disturbances in behaviour or general health were noted following repeated dermal treatment with endosulfan. No abnormal clinical signs were noted in the five females at 27 mg/kg which died on days 2 and 6 of the study. (Study report states "four" females died.p.17)

Males: One male given 9 mg/kg endosulfan died on day 5 with no visible signs of toxicity and one died on day 8 with increased salivation, blood- encrusted nose, staggering gait and dyspnoea. Dissection of these animals revealed developmental defects which were most likely present before administration of the test substance (small, immature testes, livers with developmental disturbances- enlarged parenchymal cells, loss of cytoplasmic basophilia, isolated cell necroses, and frequent mitoses). Three males administered 81 mg/kg endosulfan died on days 2 and 3. Two showed no signs of toxicity and one showed classic signs of Endosulfan intoxication: tono-clonic convulsions, increased salivation, and increased respiration.

Reactions of treated skin: Males: From days 3-7 of application, dryness and desquamation of the skin were observed at most doses except for those receiving 9 mg/kg. During this period, erythema was noted in the same animals showing dryness and desquamation. The highest score for erythema noted was "2" for well defined erythema ("1" denotes very slight erythema). No examples of edema were noted. The observed irritation subsided by day 8. Females: Dryness and desquamation were noted from days 2-7 at 1, 3, and 9

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

Results: In males, reticulocyte count in the 3 mg/kg group was significantly greater than the control value, and in females thrombocyte count in the 9 mg/kg group was significantly lower than the control value. Other hematology parameters were similar to control values.

Endosulfan had no apparent effect on hematology parameters in this study.

6. Clinical chemistry: Blood samples were taken from the retrobulbar venous plexus. Animals were killed under Nembutal anaesthesia and exsanguinated. Samples were taken randomly to avoid systematic errors.

The following clinical chemistry parameters were measured:

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

Results: In males, creatinine levels were significantly less than controls at 9 mg/kg, serum cholinesterase levels were significantly lower than controls at 9 and 27 mg/kg, and brain cholinesterase levels were significantly lower than controls at 3, 9, and 27 mg/kg. There were too few survivors at 81 mg/kg to analyze results. In females, calcium levels were significantly lower than controls at 9 mg/kg, inorganic phosphate levels were significantly greater than controls at 3 mg/kg, total lipids were significantly greater than controls at 3 mg/kg, and brain cholinesterase levels were significantly lower than controls at 1, 3, and 9 mg/kg. There were too few survivors at 27 mg/kg to analyze results.

None of the clinical chemistry results demonstrates significant toxicity of the test substance: none appeared to be dose or compound related, and the differences noted were small.

7. Urinalysis: Urine was collected during the night from animals housed individually in diuresis cages from days 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:

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appearance	hemoglobin
color	bilirubin
protein	pH value
glucose	sediment

Results: No statistically significant differences between controls and treated groups in males or females were noted in urinalysis. The pH of urine in males ranged from 5.9 to 6.4 and in females ranged from 5.6 to 6.3. The urine was clear to slightly cloudy and light to dark yellow in color. Protein levels in controls and dosed animals were 0-30 mg/dl with a few isolated measurements of approximately 100 mg/dl. Hemoglobin levels were essentially zero with a few isolated measurements of 0.2 mg/dl. No glucose or bilirubin was detected in the urine of controls or dosed animals.

Urinalysis did not demonstrate any effects of endosulfan in this study.

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

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

Results: No differences in mean absolute organ weights were noted in males. At 9 mg/kg relative organ weight of testes was significantly less than controls. In females at 9 mg/kg bodyweight mean absolute spleen and adrenal weights were significantly greater than controls, and relative spleen weight was significantly greater than controls. Organ weights were not evaluated in the only surviving female.

At 9 mg/kg bodyweight in females there appears to be an effect of endosulfan on mean absolute and relative spleen weight, and on mean absolute adrenal weight. Absolute weight of these organs increased with dose, and increases became significantly different from controls at 9 mg/kg bodyweight.

9. Histopathology: The following organs and tissues were preserved for histopathology examination (fixative unspecified):

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

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Results and Discussion: The deaths of two males at 9 mg/kg were attributed to developmental defects observed at autopsy. The referenced defects were small, immature testes, and livers with developmental disturbances- enlarged parenchymal cells, loss of cytoplasmic basophilia, isolated cell necroses, and frequent mitoses. The report also states that examination of these and other animals which died between days 1-7 showed advanced autolysis and necrosis of individual cells and groups of cells. The pathological findings were not therefore considered as an immediate cause of death. Instead, death was caused by "...a study-related toxic lesion resulting in acute heart and circulation failure, accompanied by oedema or acute congestion of blood in the lungs and an agonal release of lipids in the adrenal cortex."¹ It is not evident from the report that the referenced developmental defects precluded survival of the two males which died at 9 mg/kg, as claimed in the report summary. The pathology findings do not demonstrate a dose/response relationship for mortality caused by the test substance. However, only six animals were tested at each dose level, and death of one third occurred at 9 mg/kg. Therefore, the deaths cannot be discounted even though no deaths in males occurred at 27 mg/kg. A complete account of mortality is found in the Observations section (no.1).

1. Test Report A30753, p. 23.