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

MAY 7 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Endosulfan: Review of Toxicology Studies

Caswell No. 420 HED Proj. No. 0-0465
EPA ID No. 079401 Record No. 257423

TO: K. Samek / B. Lowery, PM Team 74
Special Review and Re-registration Division (H7508C)

FROM: Whang Phang, Ph.D. W Phang 5/3/90
Pharmacologist
HFAS/Tox. Branch II/HED (H7509C)

THROUGH: K. Clark Swentzel, Section Head K. Clark Swentzel 5/3/90
and
Marcia van Gemert, Ph.D. Marcia van Gemert 5/3/90
Branch Chief
HFAS/Tox. Branch II/HED (H7509C)

The PM Team 74 has requested an evaluation of a chronic dog study, a subchronic inhalation study in rats, a chronic feeding/carcinogenicity study in rats, and 3 dermal absorption studies in rats. Some of these studies were recently submitted by the registrant and requested for review by G. LaRocca, PM (15) of Registration Division. This reviewer has discussed with Dana Pilitt, a member of PM Team 15, about the fact that there was a duplication in the request for reviewing of some of the toxicology studies of endosulfan. He suggested to me to send the data evaluation reports of those studies to him, and he would then send a copy to you. Therefore, the reviews of the studies, which are requested by both you and PM Team 15, are transmitted to PM Team 15. The data evaluation reports of the other two studies, which were not requested by PM Team 15, are attached, and the conclusions are as follows:

1). Craine, E.M., A dermal absorption study in rats with ^{14}C endosulfan, (Alternative version of study report MRID No. 400407-01). WIL Research Labs., Report No. WIL 39028. EPA MRID No. 402236-01.

Groups of male rats (4/dose) were dosed with 0.1, 1, or 10 mg/kg and exposed for 0.5, 1, 2, 4, 10, or 24 hours; percent dose absorbed was 2.2-21.6, 0.32-21.52, and 0.08-8.38, respectively. Percent dose remaining on/in skin after soap and

water wash was 62.1-56.5, 78.1-57.7, and 80.2-66.7, respectively. The results indicated significant amount of the test material remains in the skin after soap and water wash. The study was evaluated by R. Zendzian, and it was considered as acceptable. This reviewer agrees with the analysis of R. Zendzian.

2). Hollander, Weigand, and Kramer, Endosulfan-active ingredient technical (Code: Hoe 02671)I ZD 97 0003) Testing for Subchronic Inhalation Toxicity - 21 exposures in 29 days in SPF Wistar Rats. Hoechst Aktiengesellschaft, Pharma Forschung Toxikologie; Report No. 84.05939; August 15, 1984. EPA MRID No. 00147183.

This study was previously evaluated by Margaret Jones, and the data evaluation report of this study had been transmitted to PM Team 15 on July 20, 1986. This reviewer agrees with the evaluation of M. Jones.

Groups of rats (10/sex/dose) were exposed to 0, 0.0005, 0.0010, and 0.0020 mg/L air for 21 days. An additional 5 animals/dose were held for a 4-week recovery period following exposure to the test compound. The dosage levels in this study were not adequate. Some slight effects on the clinical chemistry and in hematology counts were noted, but these findings did not demonstrate significant toxicity of the test compound. This study was classified as unacceptable.

007905

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM January 23, 1990

SUBJECT: Endosulfan, Dermal Absorption Study in Male Rats

TO: Whang Phang Ph.D
Pharmacoalogist
Rev Sec II, Tox Br II

FROM: Robert P. Zendzian Ph.D. 1/23/90
Senior Pharmacologist
Health Effects Division

Action Requested

Review the following study;

A dermal absorption study in rats with ^{14}C-endosulfan, (Alternative version of study report MRID No: 400407-01, E.M. Craine, WIL Research Labs., WIL 39028, 12/11/36, MRID 402236-01

Core Clasification Acceptable

Conclusions

Rats dosed at nominal doses of 0.1, 1 or 10 mg/kg and exposed for 0.5, 1, 2, 4, 10 or 24 hours. Percent dose absorbed was 2.2-21.6, 0.32-21.52 and 0.08-8.38 respectively. Percent dose remaining on/in skin after soap and water wash was 62.1-56.5, 78.1-57.7 and 80.2-66.7 respectively.

Discussion

This study, which was completed on 12/11/86, showed that significant portions of the dose remained on the skin of male rats following the soap and water wash. Subsiquently, 11/17/88, a study was performed in female rats to determine the fate of the skin residue (Zendzian 1990). Female rats were dosed at nominal doses of 0.1, 1, or 10 mg/kg, the application site was washed at 10 hours and four rats per dose were carried for 24, 48, 72 and 168 hours. Given the respective experimental designs, the only parameter that can be compared in the two studies is the percent of dose that was removed by washing at 10 hours (Table 1).

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Table 1. Comparison of the disposition of the dose as, percent of applied dose, with a soap and water wash at 10 hours after dosing. Values are means of 4 rats. Male data from study MRID 402236-01, Female data from study MRID 410485-04.

Group/ nominal dose	Total Exposure (hours)	Males		Females	
		Wash	Absorbed	Wash	Absorbed
Group I (0.1 mg/kg)	10	15.2	7.6		
	24			30.7	22.1
	48			35.0	35.3
	72			27.7	39.0
	168			28.0	44.8
Group II (1.0 mg/kg)	10	13.8	5.77		
	24			40.8	16.1
	48			51.1	36.2
	72			42.5	28.7
	168			46.8	46.4
Group III (10 mg/kg)	10	14.1	3.86		
	24			59.7	3.8
	48			70.2	11.1
	72			65.0	12.0
	168			68.6	20.3

There are two possible reasons for the failure of the ten hour wash data to compare, 1) different methods were used for washing and 2) different sexes were used.

1) In the first, male, study the application sites were washed, after sactificing the animals, as follows;

At 0.5, 1, 2, 4, 10 or 24 hours after dosing individual rats were euthanized with nitrogen and a 4 ml blood sample removed from the vena cava. The application site skin with rubber ring and paper cover attached was removed as a unit. The paper was removed for analysis. "The rubber ring with application site was washed with 5 ml of a mild soap solution (1.0% Ivory Liquid) and three 5 ml portions of water."

In the second, female, study the aplication sites were washed on the live animals and they were followed for the extended absorption period as follows;

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.

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The Agency has data which shows that sacrificing the animal, removing the skin and then washing the skin results in significantly greater residue on/in the skin when compared with in situ washing on living animals. This observation can explain the differences between the male and female wash data.

2) Sex differences in binding and absorption. The Agency has data on two compounds that show greater residue on/in the skin and greater absorption in females rats. Since there are no 10 hour skin residue and absorption data for the females, we are unable to determine if such a situation occured in these studies.

Reference

Memo, Zendzian to Fang, re Endosulfan, Dermal Absorption Study in Female Rats, Jan 12, 1990

Attachments

DER
One-liner

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Data Evaluation Report

Compound Endosulfan

Citation
A dermal absorption study in rats with ^{14}C-endosulfan, (Alternative version of study report MRID No: 400407-01, E.M. Craine, WIL Research Labs., WIL 39028, 12/11/86, MRID 402236-01

Reviewed by Robert P. Zendzian Ph.D. 1/23/91
Senior Pharmacologist

Core Clasification Acceptable

Conclusions

Rats dosed at nominal doses of 0.1, 1 or 10 mg/kg and exposed for 0.5, 1, 2, 4, 1 or 24 hours. Percent dose absorbed was 2.2-21.6, 0.32-21.52 and 0.08-8.38 respectively. Percent dose remaining on/in skin after soap and water wash was 62.1-56.5, 78.1-57.7 and 80.2-66.7 respectively.

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

Male Crl:CD®(SD)BR from Charles River, 5 weeks of age

Experimental Design

Rats were dosed dermally as follows;

Group Number	Number Rats	Dose mg/kg	Number of Rats At Termination (hr) 0.5	1	2	4	10	24
I	24	0.1	4	4	4	4	4	4
II	24	1.0	4	4	4	4	4	4
III	24	10.0	4	4	4	4	4	4

Dosing

Radiolabeled endosulfan was suspended in the formulation blank and further diluted with water to produce the dosing suspensions. 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. On the day of 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, in all cases 100 ul or 200 ul, 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.

At 0.5, 1, 2, 4, 10 or 24 hours after dosing individual rats were euthanized with nitrogen and a 4 ml blood sample removed from the vena cava. The application site skin with rubber ring and paper cover attached was removed as a unit. The paper was removed for analysis. "The rubber ring with application site was washed with 5 ml of a mild soap solution (1.0% Ivory Liquid) and three 5 ml portions of water." The washes were combined for analysis. "The skin of the application site was dissected away, followed by dissection of the skin adhering to the rubber ring which was designated adjacent skin." Residual urine in the bladder was added to the urine collection. Liver, kidney, brain, fat and residual carcass were collected for analysis. Total urine and feces were collected for analysis. Cages were washed with ethanol.

The following samples were analyzed for each rat;

skin wash	application site	liver
filter paper	and adjacent skin	kidney
rubber ring	untreated skin	brain
	carcass	fat

Results

Table 1 summarizes the mean doses applied and their distribution.

Table 2, from the report, presents the mean concentrations of endosulfan equilivants in the tissue samples.

Discussion

This study was performed with a variation 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 variation. 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

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skin. In this study the acetone wash was performed on the day of dosing no more than 5 hours 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 effect of the loss of lipoid material on the dermal penetration of the test material.

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Table 2

Table 12. Summary: The average concentration of endosulfan equivalents in whole blood and tissues of sub-groups of four animals sacrificed after specified periods of time. Values are expressed in ng/gram of wet tissue and were calculated from the data in Tables 9, 10 and 11.

Dose group	Time of sacrifice (hrs)	Average concentration of endosulfan equivalents in the individual tissues collected and analyzed				
		Liver (ng/g)	Kidney (ng/g)	Brain (ng/g)	Fat (ng/g)	Blood (ng/g)
I	0.5	<2	<4	<5	<12	<2
	1.0	5	7	5	<20	<2
	2.0	12	16	5	<14	2
	4.0	6	14	5	<14	<2
	10.0	13	43	5	21	3
	24.0	23	72	5	26	4
II	0.5	2	4	5	<18	<2
	1.0	23	36	5	<18	2
	2.0	46	72	8	<18	6
	4.0	78	137	10	35	10
	10.0	128	278	8	88	16
	24.0	218	710	12	210	38
III	0.5	32	46	14	<85	<8
	1.0	109	159	25	92	13
	2.0	212	444	39	95	25
	4.0	462	1146	56	256	71
	10.0	892	2790	68	797	121
	24.0	838	3446	56	994	184

MIL Analytical Research Report 38:15. Table 12. page 46

Table 1. Mean disposition of the dose. Values are the means of four male rats.

Exposure (hrs)	Actual Dose (ug/rat)	Actual Dose (ug/cm^2)	Skin Wash (ug)	Skin Wash (%)	Skin Residue (ug)	Skin Residue (%)	Total Tissues[1] (ug)	Carcass (ug)	Urine (ug)	Feces (ug)	Absorbed (ug)	Absorbed (%)
Group I (0.1 mg/kg)												
0.5	23.6	2.1	3.15	13.4	14.65	62.1	<0.05	<0.29	0.16	<0.01	0.51	2.2
1	23.7	2.2	8.40	35.4	14.46	61.0	0.11	0.21	<0.10	<0.01	0.43	1.8
2	23.5	2.2	6.09	25.9	14.69	62.5	0.19	0.42	0.12	<0.01	0.71	3.0
4	25.5	2.4	5.18	20.3	18.43	72.3	0.14	0.31	0.01	<0.01	0.47	1.8
10	25.5	2.4	3.88	15.2	17.25	67.6	0.36	1.41	0.16	<0.01	1.94	7.6
24	24.9	2.3	3.72	14.9	14.08	56.5	0.55	2.12	0.68	2.02	5.37	21.6
Group II (1.0 mg/ kg)												
0.5	150.6	13.9	28.93	19.2	117.57	78.1	0.07	<0.33	<0.07	<0.01	0.48	0.32
1	217.9	20.2	40.13	18.4	174.75	80.2	0.43	0.74	<0.07	<0.01	1.25	0.57
2	187.0	17.3	43.62	23.3	147.17	78.7	0.81	1.83	0.04	<0.01	2.69	1.44
4	242.4	22.4	58.25	24.0	173.24	71.5	1.41	4.61	0.25	<0.01	6.28	2.59
10	221.8	20.5	30.50	13.8	151.13	68.1	2.23	9.40	1.12	0.04	12.79	5.77
24	221.7	20.5	30.54	13.8	128.22	57.8	4.96	21.03	5.27	16.46	47.72	21.52
Group III (10 mg/kg)												
0.5	3053.8	282.8	419.41	13.7	2449.07	80.2	0.62	1.41	<0.35	----	2.38	0.08
1	2765.3	256.0	488.88	17.7	2039.85	73.8	1.74	3.25	<0.35	<0.01	5.35	0.29
2	2875.7	266.3	655.29	22.8	2100.02	73.0	3.64	9.58	0.43	----	13.92	0.47
4	2565.9	237.6	658.34	25.7	1715.29	66.8	7.72	24.38	2.13	<0.01	34.24	1.33
10	2161.1	200.1	304.16	14.1	1549.56	71.7	16.25	58.35	8.12	0.64	83.36	3.86
24	2173.2	201.2	295.46	13.6	1450.64	66.7	21.57	80.17	24.05	56.31	182.10	8.38

SECTION HEAD

007905

005315

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

MEMORANDUM

JUL 20 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of Subchronic Inhalation Study in Rats with Endosulfan

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

FROM: Margaret L. Jones
Review Section III
Toxicology Branch (TS-769)

THROUGH: Marcia Van Gemert, Ph.D., Head
Review Section III
Toxicology Branch
M. van Gemert 7.18.86

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

Compound: Endosulfan

Tox Chem No: 420

Registration No. 154123

Registrant: American Hoechst

Accession No: 256115, 256126

Tox. Project No: 489
MRID No. 00147183

Action Requested: Review the Subchronic inhalation study in rats which was submitted as follow-up data to the 1982 Endosulfan Registration Standard. Data on subchronic inhalation were listed as a data gap.

Conclusions: Ten male and 10 female SPF Wistar rats were admininstered 0, 0.0005, 0.0010, and 0.0020 mg Endosulfan/l air for ~~29~~ days. Air and vehicle controls were used. An additional 5 animals at each dose were held for a 4-week recovery period after receiving the test aerosol. The study apparently did not reach a maximum tolerated dose. Some slight effects on clinical chemistry and in hematology counts were noted but these do not demonstrate significant toxicity of the test compound.

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Classification: Core Unacceptable

Discussion of Selected Results and Deficiencies: Body weight in males was lowered 3-5% at the high dose from day 20-30. Lowered body weight was more pronounced in the high dose recovery group of 5 animals from day 31-60. However, since only 5 animals were held, the 12-14% lowering of body weight does not demonstrate significant toxicity and cannot be analysed statistically in a meaningful way. Food consumption in males was lower than controls in the high dose group from day 20-30 and likewise in the 5 animals held through day 60. Again, the minimal lowering of food consumption in only 5 animals does not demonstrate significant toxicity of the test compound. Erythrocytes were significantly elevated in males in the low and mid dose groups at the end of exposure (29 days). This effect is apparently spurious, since it was not observed at the high dose. The changes discussed above do not demonstrate a pattern of toxicity clearly related to the test substance. In addition, the test report states the values were within the norm for the strain and species used, although no historical control data were included to support this statement.

Specific deficiencies in the test report are listed below:

1. The study fails to demonstrate a maximum tolerated dose for the test compound with clear signs of toxicity at the high dose.

2. Length of administration of test substance was not clearly stated in the test report. There are several indications the length of administration was 21 days although most of the information indicates it was 29. Since most of the results discussed above were observed between days 20-30, the length of administration is critical to evaluation of this study.

3. Statements about monitoring of temperature and humidity were misleading. The report states these parameters were monitored "continuously", however evidence in the tables indicates measurements were taken every 5-6 days for each group.

4. Historical control data are necessary to support the statements which indicate erythrocyte counts and other parameters were normal for the species and strain considered.

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

Secondary reviewer: Marcia Van Gemert, Ph.D. M. Van Gemert 7.18.86
Section III, Tox. Branch (TS-769)

DATA EVALUATION REPORT

Study Type: 21 Day Inhalation in the Rat Tox. Chem. No.: 420

Accession Number: 256115, 256126 MRID: none
00147183

Test Material: Endosulfan

Synonyms: Thiodan®, Thionex®

Study Number(s): Report No: 84.0539 (Translation of Study No. 83.0103); [Doc. No. A 29823 (Translation of Doc. No. A29766)]

Sponsor: American Hoechst

Testing Facility: Hoechst Aktiengesellschaft, Pharma Forschung Toxikologie, Frankfurt, W. Germany

Title of Report: Endosulfan-active ingredient technical (Code: HOE 02671 OI ZD 97 0003) Testing for Subchronic Inhalation Toxicity - 21 exposures in 29 days in SPF Wistar Rats

Authors: Hollander, Weigand, Kramer

Report Issued: 15 August 1984

Conclusions: Ten male and 10 female SPF Wistar rats were administered 0, 0.0005, 0.0010, and 0.0020 mg Endosulfan/l air for 29 days. Air and vehicle controls were used. An additional 5 animals at each dose were held for a 4-week recovery period after receiving the test aerosol. The study apparently did not reach a maximum tolerated dose. Some slight effects on clinical chemistry and in hematology counts were noted but these do not demonstrate significant toxicity of the test compound.

Classification: Core Unacceptable

Discussion of Selected Results and Deficiencies: Body weight in males was lowered 3-5% at the high dose from day 20 through 30. Lowered body weight was more pronounced in the high dose recovery group of 5 animals from day 31-60. However, since only 5 animals were held, the 12-14% lowering of body weight does not demonstrate significant toxicity and cannot be analysed statistically in a meaningful way. Food consumption in males was lower than controls in the high dose group from day 20 through 30 and likewise in the 5 animals held through day 60. Again, the minimal lowering of food consumption in only 5 animals does not demonstrate significant

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toxicity of the test compound. Erythrocytes were significantly elevated in males in the low and mid dose groups at the end of exposure (29 days). This effect is apparently spurious, since it was not observed at the high dose. The changes do not demonstrate a pattern of toxicity clearly related to the test substance. In addition the test report states the values were apparently within the norm for the species and strain studied, although no historical control data were submitted to support this statement. Specific deficiencies in the test report are discussed below:

The following deficiencies were found in the test report:

1. The study fails to demonstrate a maximum tolerated dose for the test compound with clear signs of toxicity at the high dose.

2. Length of administration of test substance was not clearly or consistently stated in the test report. There are several indications the length of administration was 21 days although most of the information indicates it was 29. Since most of the results discussed above were observed between days 20-30, the length of administration is a critical factor in the evaluation of this study.

3. Statements about monitoring of temperature and humidity were misleading. The report states these parameters were monitored "continuously", however evidence in the tables indicates the measurements were taken every 5-6 days for each group.

4. Historical control data are necessary to support the statements which indicate erythrocyte counts and other parameters were normal for the species and strain considered.

A. Materials:

1. Test Compound: Endosulfan (6,7,8,9,10,10-Hexachloro-1,5,5a, 6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide); Brown flakes; Batch No. 002671 OI ZD 97 0003; 97.2% pure; contaminants not listed; Vehicle: Ethanol:polyethylene glycol 400 (1:1) administered with test substance and alone to vehicle controls; a one percent solution of the test substance with vehicle was prepared daily.

2. Test Animals: Wistar Rats, strain HOE: WISKf(SPF71) from Hoechst AG, Pharma Forschung Toxikologie, Kastengrund, bred under "SPF" conditions; 5-7 weeks old at start of test substance administration; Males weighed 114-135 g., Females weighed 114-130 g.

B. Study Design:

1. Animal assignment: Animals were assigned randomly to the

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toxicity of the test compound. Erythrocytes were significantly elevated in males in the low and mid dose groups at the end of exposure (29 days). The changes do not demonstrate a pattern of toxicity clearly related to the test substance. In addition the test report states the values were apparently within the norm for the species and strain studied, although no historical control data were submitted to support this statement. Specific deficiencies in the test report are discussed below:

The following deficiencies were found in the test report:

1. The study fails to demonstrate a maximum tolerated dose for the test compound with clear signs of toxicity at the high dose.

2. Length of administration of test substance was not clearly or consistently stated in the test report. There are several indications the length of administration was 21 days although most of the information indicates it was 29. Since most of the results discussed above were observed between days 20-30, the length of administration is a critical factor in the evaluation of this study.

3. Statements about monitoring of temperature and humidity were misleading. The report states these parameters were monitored "continuously", however evidence in the tables indicates the measurements were taken every 5-6 days for each group.

4. Historical control data are necessary to support the statements which indicate erythrocyte counts and other parameters were normal for the species and strain considered.

A. Materials:

1. Test Compound: Endosulfan (6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide); Brown flakes; Batch No. 002671 OI ZD 97 0003; 97.2% pure; contaminants not listed; Vehicle: Ethanol:polyethylene glycol 400 (1:1) administered with test substance and alone to vehicle controls; a one percent solution of the test substance with vehicle was prepared daily.

2. Test Animals: Wistar Rats, strain HOE: WISKf(SPF)(1) from Hoechst AG, Pharma Forschung Toxikologie, Kastengrund, bred under "SPF" conditions; 5-7 weeks old at start of test substance administration; Males weighed 114-135 g., Females weighed 114-130 g.

B. Study Design:

1. Animal assignment: Animals were assigned randomly to the following test groups and housed in groups of 5 animals per cage between exposures and one per cage during exposures:

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Test Group and Dose	29 admin. in 29 days		29 admin. + 4 week recovery	
	male	female	male	female
Air Control (0)	10	10	5	5
Vehicle Control (2 ml/hr)	10	10	5	5
Low (0.0005 mg a.i./l.air)	10	10	5	5
Mid (0.0010 mg a.i./l.air)	10	10	5	5
High (0.0020 mg a.i./l.air)	10	10	5	5

After apparently receiving 29 exposures in 29 days, 10 animals per group were sacrificed and 5 were held for an additional 4-week observation period. The animals sacrificed at 30 days were designated at the start of the study.

2. Exposure: Animals were exposed 6 hours/day, 5 days/week for a total of 21 (29) exposures (see comments below) by placing them "nose-only" in an 80 liter glass and stainless steel cylinder. The chamber stood in a vent pipe of 4 cubic meters volume with suction at the bottom to draw off excess aerosol.

Comment on length of exposure: The test report indicates three different lengths of exposure: the title states "21 exposures in 29 days"; p. 12 states "29 exposures in 29 days"; 22 readings were recorded during the exposure period. This review will refer to 29 exposures as the apparently correct length of time from the results section.

3. Animals received Altromin R1324 pellets and water ad libitum, except during exposures.

4. Statistics: various methods were used to analyse the different data collected in this study: body weights- parametric method of Dunnett, and distributed-free method by Nemenyi/Dunnett; hematology- the above methods and parametric method by Sidak

5. Quality Assurance inspections were performed on both Report No. 83.0103 and its translation, No. 84.0539. Reports were made 4 times between 29 April 1983 and 21 September 1984.

C. Measurements:

All measurements were made in the breathing zone of the animals. Aerosol generation was performed under dynamic conditions. Air was provided at 800 l/hr by an air-calibrated rotameter. There were 10 air changes per hour. Slight negative pressure in the chamber was maintained by drawing out test atmosphere at the rate of 1100 l/hr. Nozzles of varying diameter were used to attain the concentrations of test substance: 0.1 mm for vehicle control, 0.3 mm for low dose, and 0.15 mm for mid and high dose groups. A one percent solution of the test substance was injected into the nozzle at a constant speed. Air flow was 3 l/min (1.25 m/sec). The measurement method for air flow was not specified.

a. Gravimetric concentration was measured using Sartorius Membranfilter GmbH (Gottingen) membrane filters 50 mm. in diameter with pore width 0.65 um. once daily for vehicle controls and 3 times per day for the exposure groups, at the same time CO, CO_2, and O_2 were monitored.

b. Chemical concentration was measured by passing 18 liters of test atmosphere in 1 hour through gas washing bottles filled with acetone and placed in a cold methanol bath. Concentration of active ingredient was determined using gas chromatography. Samples were measured on days 2, 7, 14, 21, and 28.

c. Particle size was measured using a Model 225 Particle Counting System (Kratel GmbH, Gerlingen) once per hour in vehicle controls and in the exposure groups.

d. CO, CO_2, O_2, temperature, and humidity- CO, CO_2, and O_2 were measured at 30 minutes, 2 hours, and 4 hours during each exposure day. For the air only control group, these measurements were made on days 3 and 27 only. CO and CO_2 were measured with Uras 2T Infra Red Gas Analyser, O_2 with a Magnos 3 oxygen analyser, temperature with a CMR Messumformer TEU 320, and atmospheric humidity with a Vaisala HMT 12 Transmitter.

Results:

a. Gravimetric concentration:
0.0005 mg a.i. = 44 mg of 1% solution/m^3 air
0.0010 mg a.i. = 84 mg of 1% solution/m^3 air
0.0020 mg a.i. = 167 mg of 1% solution/m^3 air
vehicle control = 246 mg ethanol-polyethylene glycol 400/m^3 air

b. Chemical concentration: mean concentrations corresponding to the "ideal" ones above were:
0.00053 mg a.i. (for 0.0005 mg a.i. group)
0.00088 mg a.i. (for 0.0010 mg a.i. group)
0.00221 mg a.i. (for 0.0020 mg a.i. group)

c. Particle size: In all test groups, more than 90% of the particles were under 6 um in diameter. More than 87% of particles in the vehicle control group were under 6 um in diameter.

d. CO, CO_2, O_2, temperature, humidity: CO levels were 0-7 ppm; CO_2 ranged from approximately 3500 ppm-20000 ppm (3800-17000 for test groups, 5500-20000 for vehicle control, and 9600-11000 for air only control); O_2 content was close to 19% in test groups and air-only controls, and ranged from 18-20 in vehicle controls. Temperature was approximately 21-25°C*. Humidity* ranged from 31-54% in test groups, 28-53% in vehicle control, and 37-43% in air-only controls.

* Temperature and humidity were monitored 3 times per day on 5-6 alternate days of the 29 day test period (appproximately every 5 days for each group).

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2. Observations for toxicity and mortality: Animals were inspected before the start of the test period, during exposure periods, and at the end of the study. They were examined weekly for neurological disturbances, changes in opacity of refracting media, lesions of the oral mucosa, and abnormalities in dental growth.

   Results: No visible effects of the test compound were apparent upon observation of the majority of animals. One male in the high dose group demonstrated toxic signs consisting of emaciation, pale skin, squatting position and high-legged position from day 12 until the end of the study. No mortality occurred in the test period or in the recovery period.

3. Body weight was recorded twice weekly throughout the study. Ten animals from each group were sacrificed one day after the last exposure. The exact day of sacrifice of the original test group is not clear in the test report (day 22 or day 34, according to the information in the test report).

   Results: Group mean body weights were similar to controls with the exception of high dose males from day 20 of the exposure period to day 30, whose weights were 3-5% lower than controls. In the 5 recovery males at this dose, body weights lagged approximately 50 g. (12-16%) behind controls and other groups from day 34 to day 60. Statistical analysis of recovery results would not be meaningful due to the small sample size.

   There were apparently no compound related effects on body weight.

4. Food consumption was measured indirectly by biweekly weighing. Water consumption was measured biweekly in an unspecified manner.

   Results: Food consumption was significantly decreased in high dose males on day 20. Food consumption for the recovery group averaged from 3-4 g/day less than controls during the recovery period, except for day 34, when food consumption was 15 g less than controls. Water consumption in vehicle controls and in male dose groups was slightly increased, compared to air-only controls.

   There were apparently no compound related effects on food and water consumption.

5. Food efficiency was not reported. The calculation in only 5 animals was not considered meaningful by the reviewer.

6. Hematology parameters were examined one day after the last exposure in 10 animals from each group. Blood was withdrawn from the retrobulbar venous plexus. Parameters were again examined at the end of the recovery period in the 5 remaining animals from each group. Animals were sampled randomly to avoid systematic errors. The following parameters were measured or calculated from others:

Hemoglobin(Hb)
Reticulocytes
Hematocrit(HCT)
Leucocytes
Differential blood count
Thrombocytes
Coagulation time
Heinz bodies
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)

Results: Significant changes in erythrocytes, leucocytes, hemoglobin, and hematocrit were noted upon statistical analysis, as follows:

Subchronic Inhalation with Endosulfan in Rats/Hematology

Parameter	Sex	Control (air)	Control 2 (vehicle)	0.0005 mg/ l air	0.0010 mg/ l air	0.0020 mg/ l air
One Day After End of Exposure (n=10)						
erythrocytes ($10^{12}/l$)	M	7.62	7.91	8.48*	8.11*	8.11
leucocytes ($10^9/l$)	M	8.1	7.7	8.2	7.2	6.4*
	F	5.6	7.5*	7.3	6.6	7.2
hemoglobin (g/l)	M	156	163	172*	165	156
hematocrit (unity)	M	0.43	0.44	0.46*	0.45	0.45

* Statistically significant difference in parameter as compared to air-only controls at $p<0.05$.

Although differences in some of the above parameters are statistically significant, most of the above changes do not appear to be dose or compound related. Although erythrocyte counts were significantly increased at the low and mid doses, the effect is apparently spurious, since it was not observed at the high dose. The decrease in leucocyte count in males seems to be marginally dose related. None of the changes appears to demonstrate significant toxicity of the test compound.

7. Clinical chemistry parameters were examined one day after the last exposure and at the end of the recovery period (29 more days). Blood was sampled from the retrobulbar venous sinus. Animals were then killed by cervical dislocation under Nembutal anaesthesia and exsanguinated. The following parameters were examined:

sodium
potassium
bilirubin, total and direct
creatinine
glucose
urea nitrogen
calcium
chloride
SGOT
SGPT
AP
cholesterol
total protein
methemoglobin
lactate dehydrogenase (LDH)

Results: Significant changes at the end of the exposure period in chloride, urea nitrogen, cholesterol, calcium, creatinine, and SGOT, and at 29 days after the end of exposure in glucose, urea nitrogen, protein, LDH, and potassium were noted upon statistical analysis, as follows:

Subchronic Inhalation with Endosulfan in Rats/Clinical Chemistry

Parameter	Sex	Control (air)	Control (vehicle)	0.0005mg/l air	0.0010mg/l air	0.0020mg/l air
One Day After End of Exposure (n=10)						
chloride mmol/l	M	101	103	103*	103	103
	F	108	109	109	105*	106
urea nitrogen mmol/l	M	6.46	8.62*	8.11*	7.87	7.42
	F	7.33	8.29	7.08	6.43	8.57
cholesterol mmol/l	M	1.83	1.47*	1.62	1.73	1.51
calcium mmol/l	F	2.66	2.61	2.65	2.66	2.55*
creatinine umol/l	F	48	52	49	51	58*
SGOT U/l	F	146	137	123	134	112*

* Statistically significant difference in parameter as compared to air-only controls at $p<0.05$.

Clinical Chemistry (cont'd)

Parameter	Sex	Control (air)	Control (vehicle)	0.0005mg/ l air	0.0010mg/ l air	0.0020mg/ l air
29 Days After End of Exposure (n=5)						
glucose mmol/l	M	8.23	8.22	11.30*	9.14	8.91
urea nitrogen mmol/l	M	8.08	7.78	8.62	10.06*	9.26
	F	7.66	10.50*	9.18	8.66	8.22
protein g/l	M	59	55	53*	55	52*
LDH U/l	M	69	48	92*	145**	58
potassium mmol/l	F	5.2	5.8*	5.3	5.3	5.4

* Statistically significant difference in parameter as compared to air-only controls at $p<0.05$.
**Standard deviation = 76 (3-6 times s.d. in other groups), this value appears spurious

The changes showing statistical significance are apparently within the biological norm for this strain and species. The increase in urea nitrogen appears more related to the vehicle than to the test substance. However, there is no conclusive evidence in this regard. The observed changes do not demonstrate significant toxicity of the test compound.

8. Macroscopic examination of skin, orifices, eyes, teeth, oral mucosae, and internal organs followed sacrifice. The following organ weights were recorded: heart, lungs, liver, kidneys, spleen, brain, testes without epididymides/ ovaries, adrenals, pituitary, thyroid, seminal vesicles. As organs were prepared for histopathological examination, macroscopic findings were noted.

   Results: No dose or compound related differences between controls and dose groups were noted at macroscopic examination one day after the last administration of test substance or at examination after the recovery period. Mean absolute and relative organ weights of test groups were similar to controls at both examinations.

9. Ten males and 10 females from each group were killed one day after the last treatment. The remaining animals were killed after a 4-week recovery period. Histopathological examination of 150 rats was performed. The following organs were preserved in "fixing fluid" for microscopic examination:

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	trachea
oesophagus	ileum	salivary glands (parotid, submandibular)
duodenum	rectum	
caecum	spinal cord	diaphragm
skeletal muscle	aorta	lymph nodes (cervical, iliac)
sciatic nerve		
nose with turbinalia		

Results: Several histopathological findings occurred equally in controls and in treatment groups. These were sporadic peribronchial lymphocytic aggregates and aspiration of blood in the lung; slight periportal deposition of fat in the liver; and no haemosiderin storage in the spleen. Other findings are summarized in the following table. No dose or compound related changes were produced by the test compound, according to the histopathological report.

Subchronic Inhalation with Endosulfan in Rats/Histopathology of Organs

	Control (air)		Control (vehicle)		Low		Mid		High	
	M	F	M	F	M	F	M	F	M	F
Liver										
a)light color	1	3					2			
b)surface milky			1		2	3		1	1	1
Testes										
small			1						1	
Kidney										
a)renal pelvis dilated		1		2				1(R)		1
b)hydro-nephrosis						1				
Spleen										
light color				1						1
Ovary										
enlarged						1(R)				

(R)=recovery findings