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

010176

APR 21 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlorothalonil - Dermal Absorption Metabolism - Rat ($ none)

DP Barcode: D184858 **Case:** 819269
Submission: S429741 **Chemical No.:** 081901
MRID No.: 00147962 **TRIAD No.:** 470025-025

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and

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REQUEST: Review a rat dermal absorption metabolism study with Chlorothalonil.

Registrant: SDS Biotech Corporation

CONCLUSIONS:

Radioactive chlorothalonil was applied as a single dose to the skin of male rats at 5 mg/kg/4 ml (average of 46.7 $\mu g/cm^2$) with absorption and elimination being monitored for 120 hours.

The following conclusions resulted from the data:

1. The radioactive test article acted as an infinite dose.

2. There was a constant rate of absorption (73.2 μg-eq/day when applied as 1167 $\mu g/rat/25\ cm^2$ or 5 mg/kg).

3. Increased absorption would not be expected at doses greater than 5 mg/kg.

4. A dose of 3 mg/kg (amount on the skin after 96 hours) would also act as an infinite dose for at least one day (96 and 120 hour absorptions were both 73.2 µg-eq/day).

Classification: **Core Supplementary** - This study provides scientific data, but does not fulfill a specific Guideline. It is considered to be a "special study."

This study does not satisfy any Guideline Requirement for a Metabolism study in rats.

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy 4/15/93
Section IV, Tox. Branch II

Secondary reviewer: Elizabeth A. Doyle, Ph.D. E.A. Doyle 4/16/93
Section IV, Tox. Branch II

DATA EVALUATION REPORT

STUDY TYPE: Metabolism, Dermal Absorption - Rat (§ none)

TEST MATERIAL: ^{14}C-Chlorothalonil (^{14}C-DS-2787)

DP Barcode: D184858	**Case:** 819269
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MRID No.: 00147962	**TRIAD No.:** 470025-025

STUDY NUMBER: 649-4AM-84-0010-001 (Document No.)

SPONSOR: SDS Biotech Corporation

TESTING FACILITY: Concord Woods Laboratory
SDS Biotech Corporation
Painesville, OH 44077

TITLE OF REPORT: Study of the Dermal Absorption of ^{14}C-Chlorothalonil (^{14}C-DS-2787) by Male Rats

AUTHORS: J.P. Marciniszyn, M.C. Savides, J.C. Killeen, Jr. and J.A. Ignatoski

REPORT ISSUED: December 26, 1984

CONCLUSIONS:

Radioactive chlorothalonil was applied as a single dose to the skin of male rats at 5 mg/kg/4 ml (average of 46.7 $\mu g/cm^2$) with absorption and elimination being monitored for 120 hours.

The following conclusions resulted from the data:

1. The radioactive test article acted as an infinite dose.

2. There was a constant rate of absorption (73.2 μg-eq/day when applied as 1167 μg/rat/25 cm^2 or 5 mg/kg).

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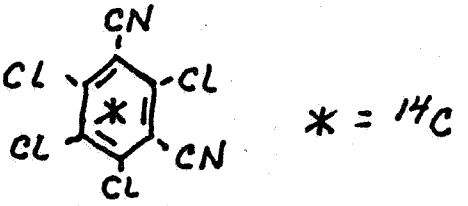
010176

I. MATERIALS AND METHODS

A. Test Article Description

Name: ^{14}C-Chlorothalonil; ^{14}C-DS-2787; Chlorothalonil (DS-2787)

Formula:



Storage: -30°C; in the dark
Composition: Radiolabelled - 117.4 mCi/mmole; purity = 99%; label in benzene ring
Nonlabelled - purity = 99.7%

B. Purity of Test Article

Report page 14 states, "The ^{14}C-radiochemical purity as determined by HPLC was 99%." "The nonlabelled material was analytical grade chlorothalonil with 99.7% purity." NOTE: No analytical data regarding either radiolabelled or nonlabelled material purity were included in the Report.

DS-2787-0503 (Analytical Grade) - SDS-Biotech Corporation

^{14}C-DS-2787 - ICN Chemical and Radioisotope Division, 2727 Campus Drive, Irvine, CA 92715

C. Animals

Male CD Sprague-Dawley rats were received from Charles River Breeding Laboratories, Inc., Portage, MI. At time of dosing, the weight range was 216-249 g. Acclimation was for at least 7 days. During acclimation, the animals were individually housed in stainless steel cages. While on study they were in metabolism cages. Room temperatures and humidity were 70-76°F and 45-52%, respectively. There was a 12 hour light/dark cycle. Ground food and water were available ad libitum.

D. Study Design

The study was conducted with a mixture of ^{14}C-radiolabelled and nonlabelled analytical grade chlorothalonil dissolved in acetone.

There was a single dermal application of 5 mg/kg/4 ml (one rat received 5 mg/kg/3 ml). Rats were given an average

of 46.7 $\mu g/cm^2$ of skin surface. Three controls received no treatment but provided samples for the determination of combustion efficiency.

Animals were assigned to the treatment group randomly (if rats weighed near 250 g the day prior to dosing, they were replaced with lighter ones, "in order to keep within the body weight range specified in the protocol (Report page 18)"). Each rat was administered about 112 μCi of ^{14}C-DS-2787.

E. Animal Preparation

The backs of the rats had the hair clipped (40 cm^2) about 24 hours pre-dosing. The clipped area was washed with acetone. Before the dosing solution was applied, a 25 cm^2 area of skin was outlined with tape and a marker.

F. Dose Preparation

^{14}C material and nonlabelled test article. The dosing solution was a mixture of a stock solution of

Purification of ^{14}C-Chlorothalonil

Report pages 19 and 20 describe, in detail, purification of the labelled material.

Specific Activity/Purity

Specific activity was determined by Gas-Liquid Chromatography (GLC) and Liquid Scintillation Counter (LSC); it was determined to be 117.43 ± 1.50, mCi/mmole (duplicate aliquots). The purity of radiolabelled chlorothalonil was determined by High Pressure Liquid Chromatography (HPLC) and LSC (Report page 20) and was determined to be 99%.

Stock Dosing Solution

To 86.3 ml of a solution of about 8.11 mCi (18.37 mg) of ^{14}C-DS-2787 (acetone) was added 71.69 mg of nonlabelled DS-2787. This was made up to 100 ml with acetone and was the stock solution.

GLC and LSC were used to ascertain the concentration, radioconcentration and specific activity of the stock solution. There were 0.758 mg of chlorothalonil/ml (stock solution), 161,959 dpm/ml and a specific activity of 25.60 mCi/mmole.

6

Dosing Solutions

Aliquots of stock solution were evaporated to dryness for storage. Before dosing, 6 ml of acetone were added to the dry samples to prepare a dosing solution of 1.26 mg of chlorothalonil/ml. [One rat received a 1.52 mg/ml solution due to insufficient volume of the original dose which remained after the 1st 5 rats were dosed.] As the volume applied dermally was based on body weight and the rat received 5 mg/kg (25 cm^2), the exposure conditions were the same as for the others in the group.

G. Animal Dosing and Route of Administration

A Hamilton syringe was used to deliver the appropriate volume of ^{14}C-chlorothalonil over the 25 cm^2 of skin. A non-occlusive patch was used to cover the treated area so that test material would not be lost. A template/screen (neoprene rubber and wire mesh screen) was glued to the back of each animal to prevent preening or interfering with the dosed area.

H. Exposure

Groups of 3 rats were exposed to the dermally applied material for 2, 4, 8, 12, 24, 48, 72, 96 or 120 hours. The animals were observed at least once daily during exposure.

I. Sample Collection

Blood

At the end of the exposure period, samples were obtained from the abdominal aorta (under ether anesthesia). Aliquots of blood were combusted and trapped CO_2 was measured by LSC to determinine radioactivity.

Urine

Samples were obtained at termination from rats exposed for 24 hours or less. Urine was collected at 24 hour intervals from animals exposed for 2-5 days. LSC was used to assay for radioactivity.

Feces

Samples, collected on the same time schedule as urine samples, were ground with dry ice (mini blender) and assayed for radioactivity by combustion and CO_2 counting (LSC).

Non-Occlusive Patch

After removal, the patches were extracted with 100 ml of acetone and aliquots assayed by LSC.

Skin

At termination, about 40 cm^2 of treated and adjacent skin were removed. This was stretched across a funnel and the radiolabelled material removed by an acetone wash of known volume. Frozen (-40°C) pieces of skin were blended, extracted twice with methanol and the extracts combined. Two acetone extractions were done and these were also combined. Methanol and acetone extracts were assayed (LSC) separately, with the radioactivity being that which had penetrated the skin but had not bound in the skin. Residual radioactivity (bound residues) was determined by combusting the skin which had been extracted, air dried and weighed.

J. Intestinal Tract

This was removed, divided into small intestine, large intestine and cecum and the contents from each rinsed with 50% methanol in water and assayed by LSC. After combustion, the tract (minus contents) was assayed by LSC.

K. Kidneys and Liver

After removal, they were separately minced and aliquots of each were combusted and assayed for radioactivity by LSC.

L. Carcass

After weighing, the carcass was cut into small pieces, minced with dry ice in a Hobart mincer, and, after combustion, aliquots were assayed for radioactivity by LSC.

M. Termination

Rats were anesthetized with ether and exsanguinated. After blood collection, treated skin, non-occlusive patch, kidneys, liver and carcass were stored frozen in individual plastic bags. As the feces were determined to be the major excretory route for the radiolabel, the intestinal tract and contents were analyzed separately from the carcass.

II. RESULTS

A. Administered Dose

The mean ± S.D. (3 rats/exposure time) of $^{14}C_2$-chlorothalonil applied to the skin was 46.7 ± 1.6 µg/cm^2. An average of 1.17 ± 0.04 mg corresponding to 4.98 ± 0.15 mg/kg was received by each animal with the dose containing 112.3 ± 3.9 µCi.

B. Dermal Integrity

One 72 hour animal had an unattached template. As fecal and skin radioactivity was the same as for the other rats in this exposure group, it was concluded that the dose was not ingested.

For two other animals (96-hour and 120-hour) with detached templates, it was determined that ingestion had occurred. Although total recovery did not differ from the other rats in the respective exposure groups, the radioactivity of the feces was 2-3 times higher and that of skin 0.6-0.7 times lower. Therefore, data from these two were not used to assess dermal absorption.

C. Material Balance

Table 1 (a copy of Report Table 2, page 44) presents the group mean radioactivity for carcass (carcass, liver, kidneys, intestinal tract and blood), skin (including non-occlusive patch, surface, extractable and bound residues), urine (urine and cage washes) and feces (feces and intestinal contents). Recovery data were expressed as: carcass = percentages of applied dose; skin = percentages of applied dose; urine and feces = cumulative percentages of applied dose.

The mean recovery of administered radiolabel was 75.77 ± 5.90% (not related to exposure period).

D. Blood

Table 2 (a copy of Report Table 3, page 45) shows group mean radioactivity values and percent of administered dose for blood.

There was an increase in radioactivity with exposure time up to 72 hours. The 96 hour determination showed a decrease (72 hour = ~ 30,000 DPM/ml; 96 hour = ~ 17,000); but at 120 hours, the value was ~ 30,000.

Blood radioactivity was mostly in plasma (89.2 ± 11.3%).

Table 1

RECOVERY OF RADIOACTIVITY FROM CARCASS, SKIN, URINE AND FECES IN A RAT METABOLISM STUDY WITH DERMALLY ADMINISTERED CHLOROTHALONIL

EXPOSURE TIME (HR)	CARCASS[a] (%AD)	SKIN[b] (%AD)	URINE[c] (%AD)	FECES[d] (%AD)	TOTAL (%AD)
2	1.14	76.01	0.09	0.16	77.39
	0.57	1.93	0.04	0.10	1.86
4	1.08	81.58	0.12	0.14	82.92
	0.63	7.02	0.03	0.05	6.74
8	4.74	57.94	0.33	0.84	63.85
	2.05	5.75	0.20	0.18	6.81
12	7.50	68.97	0.44	1.00	77.91
	5.66	17.65	0.23	0.26	11.63
24	1.56	70.64	1.97	4.33	78.49
	0.73	8.19	0.98	3.04	4.56
48	2.25	65.94	2.98	7.39	78.56
	1.53	17.33	1.54	4.24	10.36
72	3.90	49.05	3.86	13.48	70.29
	1.94	13.47	0.62	3.64	9.13
96	1.68	61.58	4.28	12.73	80.26
	0.36	2.54	0.20	0.32	2.70
120	3.64	44.51	6.04	18.03	72.22
	2.45	8.41	0.93	4.33	0.71

*Values are the mean above and standard deviation below; N=3 for all exposure times except for 96 and 120 hrs, where N=2.

[a]Sum of recoveries from carcass, liver, kidney, intestinal tract and blood.

[b]Sum of recoveries from the section of skin to which the dose was applied.

[c]Sum of recoveries from cumulative urine samples and cage wash.

[d]Sum of recoveries from cumulative feces samples and intestinal contents.

Table 2

RADIOACTIVITY IN BLOOD FOLLOWING DERMAL EXPOSURE TO CHLOROTHALONIL IN RATS

EXPOSURE TIME (HR)	BLOOD CONCENTRATION (DPM/ml)	(ng eq/ml)	% OF ADMINISTERED DOSE (%)
2	2023	9.47	0.01
	786	3.68	0.00
4	2157	10.10	0.01
	435	2.04	0.00
8	6399	29.95	0.04
	559	2.62	0.00
12	5258	24.62	0.03
	1782	8.34	0.01
24	13785	64.53	0.08
	5418	25.37	0.03
48	18006	84.29	0.11
	11271	52.76	0.07
72	29868	139.82	0.18
	12977	60.75	0.08
96	16564	77.54	0.10
	667	3.13	0.00
120	30196	141.36	0.18
	13499	63.19	0.08

*Values are the mean above and standard deviation below; N=3 for all exposure times except for 96 and 120 hrs, where N=2.

11

E. Liver and Kidney

No concentration peak was noted for either organ. The liver concentration-time curve was similar to that in blood and appeared to have plateaued. Concentrations in the liver were greater than in blood at 2, 4, 8 and 12 hours, but those in blood were greater than liver at later exposures. A plateau between 72 and 120 hours was reached in the kidney. These concentrations were greater than in blood or liver.

F. Carcass

The amount of radioactivity ranged from 1.08-7.50% of that applied with no relevance to time of exposure (Table 1).

G. Urine

Urinary radioactivity (Table 1) was cumulative (% of applied activity) so that about 1.19% of the dermal dose was excreted at 1 hour. After 120 hours, 6.04% was measured from urine plus cage washings.

H. Feces

This was the primary route of excretion with about 18% being noted after 120 hours.

I. Absorbed Dose

The amount of radioactivity in urine, feces and carcass was calculated to yield the amount of dose absorbed at each exposure interval. Table 3 presents observation data.

Table 3

ABSORPTION OF CHLOROTHALONIL FOLLOWING DERMAL APPLICATION

Exposure Time (Hr)	Amount Absorbed (µg)	% of Adminis. Dose Absorbed
2	16	1.4
4	15	1.3
8	69	5.9
12	108	8.9
24	93	7.9
48	143	12.6
72	249	21.2
96	217	18.7
120	321	27.7

NOTE: N = 3 for all exposure times except 2 at 96 and 120 hours.
Data extracted from Report Table 5, page 47.

The mean rate of absorption was calculated to be 73.2 ± 15.3 (S.D.) µg of ^{14}C-chlorothalonil equivalents/day.

J. Surface, Extractable and Bound Residues

Residues from skin surface were recoveries from the non-occlusive patch plus skin washings before skin extraction. Those which penetrated the skin are the amount of applied dose that had been extracted from the skin. Residues which remained after extractions (methanol and acetone), were those which were bound in the skin. Table 4 presents surface, extractable (penetrated), bound and total skin residues.

Table 4

SURFACE, PENETRATED, BOUND AND TOTAL SKIN RESIDUES IN A DERMAL METABOLISM STUDY WITH CHLOROTHALONIL

Exposure Time (Hr)	Surface (% AD)	Penetrated (% AD)	Bound (% AD)	Total (% AD)
2	69	2.7	4.0	76
4	76	2.3	3.2	82
8	49	3.3	5.9	58
12	63	2.4	3.6	69
24	60	2.0	8.4	71
48	52	3.0	10.5	66
72	36	2.2	11.3	49
96	41	2.2	18.3	62
120	20	2.4	22.5	45

% AD = % of Applied Dose
NOTE: N = 3 for all exposure times except 2 at 96 and 120 hours.
Data extracted from Report Table 6, page 48.

Surface residue (as % of applied material) decreased with time; whereas, the amount of "bound" increased. The percent of "penetrated" essentially remained constant at about 2.5%.

Residue values for surface, penetrated and bound were presented in the Report (Table 7, page 49) as total µg and µg/cm^2. From these data, the Report stated that the dissipation half-life was calculated to be 190.5 hours and that 73% of the applied dose was present immediately after skin application. About 76% of the radiolabel was recovered from all exposure groups.

K. Skin Absorption Rate and Permeability Constant

Report page 32 presented the following equation for the calculation of a hypothetical skin absorption rate ($\mu g/hr/cm^2$):

$$r = \frac{1/2 \text{ absorbed dose } (\mu g)}{t_{1/2} \text{ (hr)}} \times \frac{1}{\text{area treated } (cm^2)}$$

The μg in urine + feces + carcass after 120 hours determined the absorbed dose. The half-life was either elimination from blood or loss from skin. The Report indicated that the blood value was from a previous study (Report No. 621-4AM-83-0013-002). This current study was used to determine the half-life value for loss from skin and to calculate skin absorption rate. Page 32 of the Report indicated that the values derived from the current study were "questionable" due to ^{14}C-chlorothalonil evaporation from the skin and an increase in bound activity while skin radioactivity decreased after 24 hours.

Skin absorption rate was calculated to be 1.07 $\mu g/hr/cm^2$ (blood half-life) and 0.034 $\mu g/hr/cm^2$ (skin half-life).

Skin permeability constant was 2.30×10^{-2} cm/hr for blood half-life and 7.23×10^{-4} cm/hr for skin half-life (skin absorption rate divided by applied dose in $\mu g/cm^2$).

Concentration on the skin was 12.9 $\mu g/cm^3$ and was calculated from the applied dose (46.7 $\mu g/cm^3$) and the fraction of dose absorbed (0.227).

The Reviewer has no comments regarding the Methods and Materials section of this Report.

A Good Laboratory Practice Compliance statement, a Quality Assurance statement and a list of Quality Assurance inspections were included.

III. DISCUSSION

About 76% of the applied radioactivity was recovered and this was not dependent upon time of exposure. As approximately nominal doses were administered, the Report Authors indicated that incomplete recovery may have been due to evaporation from the skin.

Radioactivity was noted in the blood within 2 hours indicating rapid absorption. From 4-72 hours, blood concentration increased until a plateau was reached. Radioactivity at plateau (0.18%) after a 5 mg/kg dermal dose was less than the 1.0% noted after 5 mg/kg of oral administration. Measurements of areas under the blood curves

indicated that dermal absorption during 120 hours was similar to 30 minutes of oral absorption (previous study, Report No. 633-4AM-83-0062-002) - about 28% versus 34%, respectively.

Liver concentration paralleled that in blood, reaching a plateau at <80 ng-eq/g. Kidney concentration plateaued, but at about 700 ng-eq/g.

Urinary excretion was relatively constant (about 1.19% of the applied dose/day) and was independent of blood concentration. The Report Authors indicated that urinary excretion of the test article and/or its metabolites was saturated and that this may have been due to saturation of an active secretion mechanism and/or conjugate formation. In addition, it was stated that active secretion of acidic or basic metabolites at maximal rates may have led to a constant excretion rate. Another explanation offered by the Report Authors was the saturation of a major conjugate with small molecules such as sulfate.

Fecal radioactivity paralleled that of blood. Biliary excretion was the explanation for the presence of the ^{14}C material in the feces. As urinary excretion rate and blood concentration increased with urinary excretion remaining constant, it was concluded in the Report that biliary metabolites did not appear to have a great influence on urinary excretion. The Report Authors concluded that passive kidney filtration of chlorothalonil and/or its metabolites did not take place unless: reabsorption occurred, there was urinary excretion by active secretion and there was saturation of the active secretion mechanism. Radioactivity was absorbed during the entire 120 hour study. Total μg of ^{14}C material absorbed increased as the study progressed, with the amount during days 1-5 appearing to act as an infinite dose for 5 days when applied at 5 mg/kg. The Study Authors indicated that if more than this dose was given dermally to 25 cm^2 of skin, it was not anticipated that there would be an increase in absorption. After 96 hours, 61% of the applied ^{14}C was on or in the skin indicating a level of about 3 mg/kg. As 73.2 μg-eq/day were present between 96 and 120 hours, it was suggested that if as low as 3 mg/kg was applied to 25 cm^2 of skin, the amount absorbed during the first exposure day would be 73.2 μg-eq.

Non-absorbed radioactivity was a part of the surface, extractable or bound skin residues. The amounts decreased as exposure time increased. As the penetrated residues remained constant during the 120 hours (about 2.5%), and as these were the residues available for absorption (surface and bound skin residues not considered available), the penetration plateau was attained within 2 hours (1st interval assayed) of dose application and that skin penetration was probably the rate-limiting step for ^{14}C-chlorothalonil absorption.

Using blood and skin half-lives of 5.99 and 190.5 hours, the absorption rate was calculated to be about 1.07 and 0.034 $\mu g/hr/cm^2$, respectively, with permeability constants being 2.30×10^{-2} cm/hr (blood) and 7.23×10^{-4} (skin). The Report indicated that these were questionable values because of losses due to possible skin

evaporation and bound skin residues being unavailable. Absorption in this study indicated a constant absorption rate for 1-5 days of 6.3%/day or 73.2 µg-eq/day. Plateau in blood was attained after 72 hours. At the plateau, the Study Authors assumed absorption and elimination rates were equal. The estimated skin absorption rate was reached by using 73.2 µg of ^{14}C equivalents as being absorbed and eliminated/24 hours. Therefore, skin absorption rate was assumed as 0.122 µg/hr/cm^2 with the calculated permeability constant being 2.61×10^{-3} cm/hr. There was, therefore, the suggestion of slow absorption by these male rats.

IV. CONCLUSION

Radioactive chlorothalonil was applied as a single dose to the skin of male rats at 5 mg/kg/4 ml (average of 46.7 µg/cm^2) with absorption and elimination being monitored for 120 hours.

The following conclusions resulted from the data:

1. The radioactive test article acted as an infinite dose.
2. There was a constant rate of absorption (73.2 µg-eq/day when applied as 1167 µg/rat/25 cm^2 or 5 mg/kg).
3. Increased absorption would not be expected at doses greater than 5 mg/kg).
4. A dose of 3 mg/kg (amount on the skin after 96 hours) would also act as an infinite dose for at least one day (96 and 120 hour absorptions were both 73.2 µg-eq/day).

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