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

005911

MAY 8 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Technical Chlorothalonil Fungicide, EPA Reg. No. 50534-7, Submission of Historical Control Data for Rat Tumorigenicity Study and Additional Data Applicable to Making Risk Assessments

TO: Lois Rossi, PM 21
Fungicide-Herbicide Branch
Registration Division (TS-767)

THRU: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) 4/23/87

FROM: Brian Dementi, Ph.D.
Review Section #1
Toxicology Branch/HED (TS-769) 4/23/87

The following studies submitted by SDS Biotech Corp. have been reviewed herein:

1. Histopathologic Reevaluation of Renal Tissues from Rat and Mouse Tumorigenicity Studies. Document numbers 764-5TX-85-0071-001 (3/7/86) and 764-5TX-85-0072-002 (3/7/86). Evaluation: data will be considered fully in preparation of the peer review document for chlorothalonil.

2. An In Vitro Chromosomal Aberration Assay in Chinese Hamster Ovary (CHO) Cells with Technical Chlorthalonil. Document No. 1109-85-0082-TX-002, Accession No. 264349. May 28, 1986

Evaluation: Acceptable for the Non-Activation study; Unacceptable for the Activation study

3. Five Animal Metabolism Studies

A) Identification of Metabolites in Urine and Blood Following Oral Administratin of ^{14}C-Chlorothalonil (^{14}C-SDS-2787) to Male Rats: II. Effecs of Multiple Dose Administration on the Excretion of Thiol Metabolites in Urine. Document No. 621-4AM-83-0061-002. Accession No. 264350. May 23, 1986

Evaluation: Acceptable

B) Study of the Distribution of Radioactivity Following Repeated Oral Administration of ^{14}C-Chlorothalonil (^{14}C-SDS-2787) to Male Sprague-Dawley Rats. Document No. 1173-84-0079-AM-003. Accession No. 264350. July 3, 1986

Evaluation: Acceptable

C) Study of the Biliary Excretion of Radioactivity Following Oral Administration of (^{14}C-SDS-2787) to Male Sprague-Dawley Rats. Document No. 633-4AM-85-0012-002. Accession No. 264351. May 13, 1986

Evaluation: Acceptable

D) Pilot Study to Determine the Concentration of Radiolabel in Kidneys Following Administration of the Mono-Glutathione Conjugate of ^{14}C-Chlorothalonil to Male Rats. Document No. 631-4AM-85-0064-001. Accession No. 264351. April 23, 1986

Evaluation: Acceptable

E) Study of the Distribution of Radioactivity Following Repeated Oral Administration of (^{14}C-SDS-2787) to Male Sprague-Dawley Rats.

1. Interim Report - Multiple Versus Single Dose Comparison. Document No. 631-4AM-84-0079-001. Accession No. 258776. July 15, 1985
This report is a resubmission and was previously evaluated in Toxicology Branch (2/19/86) as supplementary.

4. The Estimation of the Lifetime Tumorigenic Risk to Humans from Dietary Exposure to Chlorothalonil. SDS 2787. Document No. 1117-86-0049-TX-001. Accession No. 264352. August 12, 1986

Evaluation: To be considered by Toxicology Branch when developing risk assessment.

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Chlorothalonil: Review of Histopathologic Reevaluation of Renal Tissue from the Rat and Mouse Tumorigenicity Studies; TOX CHEM No. 215 B.

FROM: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) 12/4/87

TO: Brian Dementi, Ph.D.
Review Section #1
Toxicology Branch/HED (TS-769)

I have previously reviewed the SDS Biotech histopathologic reevaluation of renal tissue from the rat and mouse oncogenicity studies in regard to the original reviews of these studies for EPA and WHO (JMPR), as follows:

A. Histopathologic Reevaluation of Renal Tissue from a Rat Tumorigenicity Study with Chlorothalonil (5TX-80-0234), Document # 764-5TX-85-0071-002, dated 3/7/86.

Submitted to Jaeger 9/18/85 and 10/14/85.
Evaluated by Jaeger 11/20/85 for WHO.
Evaluated by Jaeger/Ritter 2/20/86 for RS data call-in.

B. Histopathologic Reevaluation of Renal Tissue from a Mouse Tumorigenicity Study with Chlorothalonil (5TX-79-0102), Document # 764-5TX-85-0072-002, dated 3/7/86.

Submitted to Jaeger 9/18/85 and 10/14/85.
Evaluated by Jaeger 11/20/85 for WHO.
Original review of study by Ritter, 3/21/81, PP#F2875.

The historical control data from IRDC are appended and will be considered fully in preparation of the Peer Review document for chlorothalonil.

Attch. #1 Historical control data.

Page_____ is not included in this copy.

Pages 5 through 10 are not included.

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 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.

√ 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.

84-2 - CHO Cells - In-Vitro Cytogenetic Assay

005911

Reviewed by: John H.S. Chen 4/9/87
Section I, Toxicology Branch (TS-769C)
Secondary reviewer: R.B. Jaeger 4/9/87
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Chromosomal Aberration

TOX. CHEM. No.: 215B

Accession No.: 264349

MRID No.:

Test Material: Chlorothalonil (T-117-12; 98.8% Purity)

Study Number(s): T-481.337

Sponsor: SDS Biotech Corp.

Test Facility: Microbiological Associates, Inc. Bethesda, MD

Title of Report: In-Vitro Chromosomal Aberration Assay in Chinese Hamster Ovary (CHO) Cells with Technical Chlorothalonil

Author(s): M. Mizens and D.L. Putman

Report Issued: May 29, 1986

Conclusions:

In the non-activation assay, positive response was observed at the dose level of 0.3 ug/ml.

Levels tested: 0.03, 0.08, 0.15, and 0.30 ug/ml.

In the activated assay, results were unacceptable.

Levels tested: 0.6, 1.5, 3, and 6 ug/ml.

Classification of Data:

Acceptable for the non-activation study and unacceptable for the activated study.

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Title of Report: In-Vitro Chromosomal Aberration Assay in Chinese Hamster Ovary (CHO) Cells with Technical Chlorothalonil

Procedure:

1. Cell Line

The Chinese hamster ovary cell line ($CHO-K_1$) was obtained from the American Type Culture Collection (Repository Number CCL 61). The CHO cells were grown in McCoy's 5A medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 ug/ml streptomycin and 2 mM L-glutamine. The CHO cell cultures were initiated by seeding 5×10^5 cells/flask and were incubated for 16-24 hours in a humidified CO_2 incubator at 37 C, prior to the treatment of test compound.

2. Metabolic Activation System

The in-vitro metabolic activation system contained rat liver enzymes and an energy-producing system necessary for their function (i.e., NADP and isocitric acid). The preparation of liver microsomes (S-9 fraction) from rats treated previously with Aroclor was based on the method described by Ames (Mutation Res. 31: 347-364, 1975). The final made up S-9 mix contained 1.4 mg NADP, 4.5 mg isocitric acid and 15 ul S-9 per ml of growth medium with 2.5% serum.

3. Chromosomal Aberration Assay

For the chromosomal aberration assay, duplicate flasks seeded with 5×10^5 cells/flask were exposed to 4 concentration of T-11T-12 (0.3, 0.15, 0.08, and 0.03 ug/ml) for 14 hours at 37 C. For the assay with metabolic activation, the cells were exposed to 4 concentrations of the test compound (6, 3, 1.5, and 0.6 ug/ml) for 2 hours at 37 C. The treatment medium was removed, the cells washed and incubated in medium for 8 hours. Two hours before harvesting the cells, colcemid (0.1 ug/ml) was added into culture vessel to arrest the cells. The mitotic cells were harvested by a mitotic shake-off procedure, then, swelled in a hypotonic solution of 0.075 M KCl at room temperature and fixed in 2 changes of an ice cold, freshly prepared Carnoy's fixative. Chromosome slides were prepared by resuspending the cells in a small volume of fixative, dropped onto a moist glass slide and and allowed to air dry overnight. The slides were stained with 5% Giemsa, air dried and permanently mounted. Fifty metaphase c[illegible] were analyzed in each duplicate treatment flask for a total of 100 cells per treatment group for the evidence of chromosome aberrations. The solvent (acetone) and positive controls were run concurrently with the test compound.

4. Statistical Analysis

Statistical analysis of the frequency of structural aberrations per cell was performed using Dunnett's t test. Chi-Square analysis using a 2 X 2 contingency table was used to ascertain significant differences between the number of cells with numerical aberrations in the treatment and control groups. The test compound was considered to induce a positive response when the number of structural aberrations per cell was significantly increased ($P<0.05$) in a dose response manner.

Results:

(1) Preliminary Toxicity Test Using T-117-12[d]

Treatment[a]	S-9	Growth Potential		Cell Cycle Kinetics		
		Cells/flask (X 10^6)	Relative Cell Growth[b] (%)	Percentage of Cells in[e] M_1	M_2	M_3
Acetone	+	2.78	100	5	94	1
T-117-12						
1000 ug/ml	+	1.09	39	0	0	0
300 "	+	0.95	34	0	0	0
100 "	+	0.95	34	0	0	0
30 "	+	1.14	41	0	0	0
10 "	+	1.23	44	0	0	0
3 "	+	2.30	83	20	79	1
1 "	+	2.95	106	14	86	0
0.3 "	+	2.91	105	13	86	1
0.1 "	+	3.00	108	9	89	2
Acetone	-	3.21	100	8	89	3
T-117-12						
1000 ug/ml	-	0.52	16	0	0	0
300 "	-	0.55	17	0	0	0
100 "	-	0.55	17	0	0	0
30 "	-	0.62	19	0	0	0
10 "	-	0.76	24	0	0	0
3 "	-	0.96	30	0	0	0
1 "	-	1.11	35	100[c]	0	0
0.3 "	-	1.33	41	94	6	0
0.1 "	-	2.49	78	42	55	3

a. CHO cells were treated in the absence of metabolic activation for 6 hours and in the presence of metabolic activation for 2 hours at 37°C.
b. Relative Cell Growth = (Cells/treatment flask X 100)/Cells/solvent flask.
c. Only three cells in metaphase.
d. The highest dose level should be selected at least 50% toxicity (reduction in mitotic index). The cell cycle kinetics study was used to determine the optimum harvest time.
e. M_1 = 1st-posttreatment metaphase stage (12 hrs); M_2 = 2nd-posttreatment metaphase stage (24 hrs); M_3 = 3rd-posttreatment metaphase stage (36 hrs) (See also Page 5 of study report).

Findings:

i. Under the nonactivation assay system, a few metaphase cells could be identified at the doses greater than 1 ug/ml. Therefore, the highest dose was selected at 0.3 ug/ml. At this dose, a sufficient number of cells in metaphase (94% in M_1) also could be used for scoring.

ii. Under the activation assay system, the MI (83%) was not sufficiently reduced to meet the protocol specification for the highest dose (50% toxicity at the dose level of 3 ug/ml. Therefore, the highest dose was selected at 5 ug/ml. However, the compound at 3 ug/ml had caused substantial

Findings: continued

cell cycle delay in cells exposed to T-117-12 (20% metaphase cells in M_1 and 79% metaphase cells in M_2) under this assay system. Since the majority of metaphase cells were identified in the 2nd-posttreatment metaphase stage (M_2), the fixation time should be adjusted accordingly. Therefore, multiple culture harvest times (12 hours: M_1 cells; 24 hours: M_2 cells) should be considered for this clastogenicity assay under the activation system. Results obtained from the treated cells at their 2nd division cycle (M_2) were not given in this study.

(2) Summary of Chromosomal Aberrations in CHO Cells

Treatment	Cell Scored	Number and Types of Aberrations Scored	Cells with Structural Aberration (%)	Structural Aberrations Per Cell
w/o S-9				
Untreated Cells	100	0	0	0
Solvent Acetone	100	0	0	0
TEM, 1 ug/ml	100	3A,3B,22F,8G, 3H,1J,3K,43L	47	1.22*
T-117-12				
0.03 ug/ml	100	1D,2L	2	0.03
0.08 "	100	1G,4L	2	0.05
0.15 "	100	3F,2G,1H,1K,3L	9	0.21
0.30 "	100	1E,9F,2G,1H,3J, 20L	22	0.63*
w/S-9				
Untreated Cells	100	0	0	0
Solvent Acetone	100	0	0	0
CP, 100 ug/ml	100	1A,8F,2G,1I,1K, 11L	17	0.42*
T-117-12				
0.60 ug/ml	100	1L	1	0.01
1.50 "	100	0	0	0
3.00 "	100	2L	2	0.02
6.00 "	100	0	0	0

* Significantly greater than the solvent control, $P<0.05$; Chromatid and chromosome gaps were not included for scoring; A=Chromosome Break; B=Chromatid Fragment; C=Acentric Fragments; D=Dicentric; E=Ring; F=Triradial; G=Quadriradial; H=Complex Arrangements; I=Pulverized Chromsomes; J=Pulverized Cell; K=Greater than 10 Aberrations/Cell; L=Chromatid break.

Findings:

i. The positive control compounds (Tristnylenemelamine and Cyclophosphamide) induced significantly elevated levels of chromosome damages in all the experiments, thus, demonstrated the sensitivity of the in-vitro cytogenetic assay in CHO cells.

Findings:

ii. Under the test conditions reported, results showed that no evidence of chromosomal aberration was observed in the presence of metabolic activation at the dose levels tested (0.6 through 6 ug/ml). However, in the non-activation study, the frequency of cells with chromosomal aberrations was significantly increased ($P<0.05$) relative to the solvent control at the high dose (0.3 ug/ml) of T-117-12 in a dose-response manner. At this dose, chromatid breaks, ring, rearrangements (triradial, quadriradial, and complex arrangements), and pulverized cells were observed in this study.

Evaluation:

1. The in-vitro cytogenetic assay in CHO cells without metabolic activation appears to have been conducted in a manner to generate valid results. Technical Chlorothalonil (T-117-12) is clastogenic in the the non-activation study at the dose level tested (0.3 ug/ml). However, Toxicology Branch agrees that the positive results without metabolic activation may be of no biological significance to the intact mammalian organisms.

2. Although no structural chromosome aberrations in CHO cells were reported in the activated study, the test results obtained only by using one harvest time (8 hours) are considered inconclusive for this study. Since the test compound caused substantial cell cycle delay under the activated system, two harvest times (12 hours for M_1 cells and 24 hours for M_2 cells) should be considered for conducting this study in order to avoid missing a peak of aberration yield (See also EPA Health Effects Test Guidelines for Conducting the In-Vitro Mammalian Cytogenetics, EPA 560/6-83-001). Therefore, this activated study is unacceptable in the present form. However, the study may be upgraded on resolution of the reporting deficiency.

005911

Reviewed by: Brian Dementi, Ph.D. Brian Dementi 3/3/87
Section I, Toxicology Branch (TS-769C)
Secondary Reviewer: R. Bruce Jaeger, Section Head
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Metabolism, Rat Tox. Chem. No.: 081901

Accession No.: 264350

Test Material: ^{14}C-Chlorothalonil

Synonyms: ^{14}C-DS-2787

Study Number: 621-4AM-83-0061-002
Report/SDS-2787

Sponsor: SDS Biotech Corporation
Painesville, OH

Testing Facility: SDS Biotech Corp., Department of Safety Assessment, Painesville, OH

Title of Report: Identification of Metabolites in Urine and Blood Following Oral Administration of ^{14}C-Chlorothalonil (^{14}C-SDS-2787) to Male Rats: II. Effects of Multiple Dose Administration on the Excretion of Thiol Metabolites in Urine

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

Report Issued: May 23, 1986

Purpose of Study:

To identify chlorothalonil metabolites in urine and to assess the effects of multiple dosing on excretion of thiol metabolites in urine.

Conclusions:

1. The pH of urine excreted by male rats was observed to increase in response to repeated administration of chlorothalonil at all doses employed. During 4 days of dosing, urine pH rose approximately 1 pH unit at doses of 50 mg/kg and less, and increased 1.7 pH units at the high dose of 160 mg/kg.

2a. Dithiodichloroisophthalonitrile and trithiochloroisophthalonitrile were positively identified as methyl derivatives in the extractable urine fraction. On day 1 of dosing, these metabolites constituted 20.9, 24.9, and 32.2 percent of the total radiolabel in urine at the 5, 50, and 160 mg/kg dose levels, respectively. These percentages decreased with repeated administration of a given dose. Hence, > 70 to 80 percent radiolabeled material in urine was not well characterized. Involvement of the glutathione pathway is considered likely.

2b. Cysteinyltrichloroisophthalonitrile and cysteinyltrichlorocyanobenzoic acid are speculated to be among those metabolites counted in the nonextractable urine fraction. Qualitative evidence for this is inadequate.

Special Review Criteria:

A. Materials:

1. Test Compound: ^{14}C-Chlorothalonil.

Description: Radiochemical purity, 97%; uniformly labeled in benzene ring.

Batch No.: N/A.

Purity: Analytical grade chlorothalonil of 99.7% purity.

Contaminants: N/A.

2. Test Animals: Species: Rat; Strain: CD Sprague-Dawley; Age: 11 to 12 weeks; Weight: 300 to 350 grams; Source: Charles River Breeding Laboratories, Portage, MI.

B. Study Design:

"Eighty male rats were assigned at random to four dosing groups. Twenty rats were dosed at each of four dose levels: 1.5, 5, 50, or 160 mg/kg on 5 consecutive days. Four rats from each dose level were sacrificed at 2, 9, 24, 96, and 168 hours after the final (5th) dose administration. Urine samples were collected from each dose group at 24-hour intervals after each dose administration.

"To obtain sufficient material for subsequent analyses, urine samples collected from several animals

on a given collection day at a given dose level were pooled. Urine which was found to be contaminated with feces was excluded from the pools. Pooled samples were appropriately labeled as to time of collection, dose level, and animal numbers.

"The volume of the pooled urine samples was measured and aliquots were taken for measurement of the amount of radiolabel by liquid scintillation counting (LSC) according to SOP #04-T106-00. The total amount of radioactivity in the filtrate was determined by multiplying the amount of radioactivity in the aliquots (as DPM per mL) by the total volume of the filtrate. The amounts of radiolabel in urine and in the filtrate were converted to microgram equivalents per sample using the total DPM/sample and the specific activity of the appropriate dosing suspension (DPM/ug).

"B. EXTRACTION

Preliminary experiments had shown that radiolabel was not effectively extracted from urine at either neutral (pH 7) or alkaline (pH 10) pH. Extraction under acidic (pH 2) conditions was found to be effective; therefore the pH of each urine filtrate was adjusted to pH 2 with 1 N HCl prior to extraction. The acidified urine filtrate was extracted three times with four volumes of ethyl acetate per volume of filtrate. (The ethyl acetate used had been saturated with 1 N HCl.) The quantity of radiolabel extracted into the ethyl acetate phase was determined by LSC and the extractability was calculated as a percent of the total radioactivity extracted into the organic phase. The three ethyl acetate extracts were combined and the number of microgram equivalents in the extracts was calculated using the amount of radiolabel (DPM) present in each extract and the specific activity of the appropriate dosing suspension (DPM/ug).

"The acidic, aqueous phase, which remained after extraction with pH 2 ethyl acetate, was designated as the nonextractable phase of urine. The volume of the nonextractable phase was measured and the amount of radiolabel was determined by LSC.

"C. SEP PAK® TREATMEMT

The combined extract was rotovapped to remove the ethyl acetate. The residue was dissolved in a known, small volume of methanol, introduced into a Water's® C-18 Sep Pak® cartridge and eluted from

the cartridge with methanol. The amount of radiolabel eluted with methnol was quantified by LSC and converted to microgram equivalents of chlorothalonil. Approximately 97 to 99% of the radiolabel eluting from the Sep Pak® was recovered in the methanol eluate.

"D. DERIVATIZATION

The methanol eluate was concentrated to near dryness under a gentle stream of nitrogen gas. Prior to methylation, aliquots from selected samples were analyzed by gas chromatography/mass spectroscopy (GC/MS). The major portion of the concentrated eluate was derivatized using diazomethane for GC/MS analyses of methylated metabolites. In some cases, N-propyltolyltriazine was used for derivatization to distinguish between groups which had been excreted as methylated derivatives and those which had not been methylated in vivo.

"A portion of the nonextractable phase of urine from animals dosed at 160 mg/kg/day was derivatized using diazomethane after hydrolysis with 12 N HCl overnight (approximately 17 hours) at 100 °C. The derivatized sample was analyzed by GC/MS (pp. 3-5)."

Results:

Urinary pH was increased in response to dosing with chlorothalonil. Increases of approximately 1 pH unit were seen following replicate doses of 50 mg/kg and less, and 1.7 pH units following repeated dosing at the 160 mg/kg dose (table 1, p. 17).

The extractability (ethyl acetate) of radiolabel following the initial dose was greatest (84%) for low dose urine samples and decreased with increasing doses to 71 percent extraction at the high dose. Furthermore, at each dose level extractability decreased progressively with replicate dosing. For example, at the 1.5 mg/kg dose, extractability declined from 84 percent to 74 percent from the first to the fifth dosing. Likewise, at the 160 mg/kg dose, extractability declined from 71 percent to 49 percent (table 2, p. 18).

GC/MS analyses were successfully conducted on samples obtained on days 1 through 4 at the 50 and 160 mg/kg dose levels, and on the day 1 sample obtained from the 5 mg/kg regimen. Practical limitations of the procedure precluded such determinations for the 1.5 mg/kg dose group.

Dithiodichloroisophthalonitrile (dithiol) and trithiochloroisophthalonitrile (trithiol) (as methyl derivatives in both cases) were identified. It should be mentioned that since methylation of the extractables using diazomethane was performed prior to GC/MS analysis, the investigators attempted to determine which methylated or unmethylated derivatives, as the case may be, were actually excreted as metabolites. Accordingly, portions of extractable fractions were reacted with N-propyltolyltriazine as a means of introducing propyl rather than methyl substituents at unmethylated thiol sites on the various metabolites. GC/MS results enabled the conclusion to be drawn that the dithiol metabolite is actually present in the extractable fraction of urine as a mixture of the monomethyl and dimethyl derivatives and that its trithiol exists in urine as a mixture of the monomethyl, dimethyl, and trimethyl derivatives of the parent molecule. For chemical names and structures of chemical entities in question see pages 10 and 21 of the petitioner's report.

The total of the two metabolites (dithiol and trithiol) quantitated in the extractable fraction of urine on day 1 represented 20.9, 24.9, and 32.2 percent of the total radiolabel in urine at the 5, 50, and 160 mg/kg dose levels, respectively. The remaining 70 to 80 percent radiolabeled material in urine was not well characterized, however, with respect to the nonextractable urine components evidence permits speculation that likely radiolabeled metabolites include cysteinyltrichloroisophthalonitrile and cysteinyltrichlorocyanobenzoic (p. 10).

As to the extractable components, table 3 (p. 19) shows that on day 1 absolute amounts of dithiol and trithiol increased with increasing dose of chlorothalonil, however as a percentage of extractable radioactive materials, day 1 samples were constant for trithiol (i.e., independent of dose) at about 16 percent, but a dose-dependent increase for dithiol was evident, i.e., 5.2 percent (5 mg/kg), 9.0 percent (50 mg/kg), and 15.4 percent (160 mg/kg). Following replicate dosing at 50 and 160 mg/kg, there were marked declines in both absolute amounts of the two thiols and their percentages of extractable radiolabeled material, with dithiol exhibiting the more dramatic decline (table 3, p. 19).

The ratio, trithiol/dithiol, as determined at the various time points is presented in the following reproduction of table 4 (p. 20) from the submitted study.

THE RATIO OF TRI- AND DITHIOLS IN URINE

Dose mg/kg/day	Day	*Ratio ug Tri/ug Di
5	1	3.00
50	1	1.77
	2	2.84
	3	3.87
	4	3.91
160	1	1.09
	2	2.31
	3	3.56
	4	52

* ug = microgram equivalents.

As revealed in this table, at day 1 the ratio was inversely related to dose. When chlorothalonil was repeatedly administered to male rats at 50 and 160 mg/kg/day, the ratio increased with each dose administered (excepting day 4 of the 50 mg/kg dose group, which appears to have plateaued at day 3). After 4 days of administration, ratios at 50 and 160 mg/kg were 4 and 52, respectively.

Discussion:

Since there was a decreasing percentage of extractable radiolabeled material with increasing dose and with replicate dosing at all dose levels Table 2 (p.18) it is reasonable to conclude that shifts in distribution favoring polar metabolites occurs. This suggests, as the study authors note, that changes occur in the metabolism of chlorothalonil as dose level increases and upon multiple dose administration.

Data presented in Table 3 (p.19) reveal marked declines in urine levels of di- and trithiols with increasing daily dosing of chlorothalonil at both the 50 and 160 mg/kg/day dose levels. A more dramatic effect was observed at the higher dose, where between days 1 and 4 of replicate dosing the urinary dithiol level declined from 1542 to 9 ug. Since the decline in dithiol was much more precipitous than that of the trithiol, the ratio, trithiol/dithiol, rose to 52 as shown in the above table. A proportionate increase in urine trithiol to that of the decrease in dithiol was not seen, suggesting the induction or enhancement of a metabolic pathway

for the dithiol which would compete with that of the proposed dithiol ----> trithiol pathway, thus markedly enhancing the urinary trithiol/dithiol ratio.

Insufficient data are available to specifically explain why the amount of dithiol excreted in the urine decreased more rapidly than the trithiol with repeated dosing at 50 and 160 mg/kg. The petitioner speculates that diversions of the dithiol or a preceeding metabolite from the glutathione pathway to a pathway producing highly polar metabolites could explain the phenomenon. It is reported that extractable fractions are undergoing further analysis to identify additional metabolites.

The dithiol and trithiol metabolites are speculated to have been derived via glutathione conjugation at two/three sites of the chlorothalonil molecule, respectively. This is supported, according to the petitioner, by another study as cited which demonstrated glutathione conjugation with chlorothalonil, in vitro and in vivo. Dithiol and trithiol metabolites accordingly would arise via enzymatic cleavage of glutathione conjugates followed by varying degrees of methylation.

Conclusions: See page 2.

Core Rating: Minimum.

005911

Reviewed by: Brian Dementi, Ph.D. Brian Dementi 3/3/87
Section I, Toxicology Branch (TS-769C)
Secondary Reviewer: R. Bruce Jaeger, Section Head 4/3/87
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Metabolism, Rat Tox. Chem. No.: 081901

Accession No.: 264351

Test Material: ^{14}C-Chlorothalonil

Synonyms: ^{14}C-SDS-2787

Study Number: 633-4AM-85-0012-002
Report/SDS-2787

Sponsor: SDS Biotech Corporation
Painesville, OH

Testing Facility: Huntingdon Research Centre,
Huntingdon, Cambridgeshire, England

Title of Report: Study of the Biliary Excretion of Radioactivity Following Oral Administration of (^{14}C-SDS-2787) to Male Sprague-Dawley Rats

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

Report Issued: May 13, 1986

Conclusions:

At low doses (1.5 mg/kg) of orally administered radiolabeled chlorothalonil, cannulation of the bile duct had little or no effect on blood levels of radiolabel for at least 24 hours postdosing. However, at higher doses (50 and 200 mg/kg) blood levels of radiolabel were substantially higher in noncannulated animals, perhaps due to reabsorption in the intact animal.

In the dose range 1.5 to 50 mg/kg, biliary excretion, in terms of the percentage of dose administered 48 hours postdosing, was essentially constant. However, at the higher dose, 200 mg/kg, the comparable biliary excretion figure was significantly reduced, suggesting saturation of the overall biliary excretion pathway.

Data on urinary excretion of radiolabel indicate that saturation via this route of elimination lies somewhere in the dosage range 5 to 50 mg/kg.

With respect to the fecal excretion there appeared to be no relationship between fecal elimination and biliary excretion.

Kidney concentrations did not appear to be affected by bile duct cannulation suggesting a lack of meaningful biliary reabsorption from the GI tract did not influence kidney levels.

Special Review Criteria:

A. Materials:

1. Test Compound: ^{14}C-Chlorothalonil.

Description: Mixture of nonlabeled and ^{14}C-labeled chlorothalonil suspended in 0.75% methylcellulose.

Batch No.: N/A.

Purity: The nonlabeled chlorothalonil was analytical grade; 99.7% purity. The ^{14}C-chlorothalonil had a specific activity of 78.8 mCi/mMole and radiochemical purity of 97.7 to 98.4 percent.

Contaminants: N/A.

2. Test Animals: Species: Rate, male; Strain: CD Sprague-Dawley; Weight: 258 to 281 grams; Source: Charles River Breeding Laboratories, Portage, MI.

B. Study Design:

"Twenty-four male Sprague-Dawley CD rats, 258 to 281 grams in body weight at time of dosing, were obtained from Charles River Breeding Labs and were allocated at random to dosing groups. The bile ducts of all rats were cannulated immediately prior to dose administration. Six rats were dosed at each of four dose levels: 1.5, 5, 50, or 200 mg/kg. The ^{14}C-chlorothalonil was orally administered as a microparticulate suspension in 0.75 percent methylcellulose.

"Bile was collected continuously in 60 minute fractions from dose administration until 48 hours post-dosing. Blood samples were collected at various times after dosing and at termination. The choice of sampling times was based upon A) the times to peak blood concentration found in a pharmacokinetic study (1) which for

5 mg/kg was 6.1 ± 1.1 hours, for 50 mg/kg was 8.9 ± 0.7 hours and for 200 mg/kg 15.9 ± 5.8 hours; B) the sampling times used in a previously conducted bile study (2) at 5 mg/kg which were 6, 24, and 48 hours and C) based upon the data from the pharmacokinetic study, which showed that the time to peak blood concentrations increased with increasing dose level, three of the sampling times at 1.5 mg/kg were chosen to be less than 6 hours after dosing.

"For animals dosed at the 1.5 mg/kg dose level, blood samples were collected from three of the rats at 2, 5, 8, and 48 hours postdose and from the other three rats at 4, 6, 24, and 48 hours postdose. At 5 and 50 mg/kg, blood samples were collected from three rats at each dose level at 2, 6, 10, and 48 hours postdose and from the other three rats at each dose level at 4, 8, 24, and 48 hours. At 200 mg/kg, blood samples were collected from three of the rats at 6, 10, 16, and 48 hours postdose and from the other three rats at 8, 12, 24, and 48 hours.

"Urine and fecal samples were collected 6, 24, and 48 hours after dosing. At termination, the kidneys were removed for separate analyses and the gastrointestinal tract was separated from each carcass. Levels of radioactivity were determined in each bile, blood, urine, fecal and kidney sample and in the tissues and contents of the gastrointestinal tract, remaining carcass and cage washes (pp. 2-4)."

Results:

1. Concentrations of Radiolabel in Blood

At the 1.5 mg/kg dose, the mean blood concentration of radiolabel for the first 2 to 6 hours was essentially constant at 74.3 ng equiv/mL, and declined steadily beyond the 6-hour time point. As compared with a similar study, cited by the petitioner, involving noncannulated animals, blood levels of radiolabel were essentially the same for periods up to 6 hours, suggesting that cannulation had no effect upon radiolabeled uptake into blood at this low dose for a 6-hour period. After 24 hours, the blood level for cannulated animals (28 ng equiv/mL) was still essentially the same as for uncannulated rats (19 ng equiv/mL). This parallel in blood levels between cannulated and uncannulated animals at the 1.5 mg/kg dose level did not prevail at higher doses, where noncannulated animals exhibited higher blood level, as one might expect from the point of view of the

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likelihood of reabsorption of radiolabeled consequent to enterohepatic circulation. Thus at 4 hours postadministration of 5 mg/kg, the time point of maximum blood concentration, the mean blood level was 264 ng equiv/mL as compared with 489 ng equiv/mL in noncannulated rats, i.e., in cannulated rats, blood levels were approximately 54 percent that in noncannulated animals.

Following the 50 mg/kg dose, the maximum blood concentration was reached in 6 hours where the mean concentration was 3180 ng equiv/mL. In non-cannulated rats, the blood concentrations at 6 hours were 2 to 2.5 times that of cannulated rats.

At the highest dose level, 200 mg/kg, there were two peaks in blood concentration, but such levels in the noncannulated rats of the present study were reported as being at least twice as high as in cannulated rats.

2. Biliary Extraction

Percentages of the administered doses excreted in bile within 48 hours of administration were reported as follows:

Dose, mg/kg	Mean Dose Percent Excreted in Bile, 48 Hours	Mean Time to Peak Bile Conc., Hours
1.5	22.5	2
5	16.4 (19.07)*	3
50	16.3	7
200	[illegible].8	25-26

*Results obtained when additional experimental results included in the average.

At all doses administered, radioactivity was measurable in bile within 1 hour. The biliary excretion of 22.5 percent of the administered dose as observed at the 1.5 mg/kg dose level was significantly different from the 16.4 percent figure following the 5 mg/kg dose. However, the study directors invoke an argument that the difference between these doses is actually not significant when data from another experiment at 5 mg/kg is averaged with data of this experiment, yielding 19.07 percent, as indicated above. The petitioner claims that for the first three doses there are no significant differences in the 48-hour excretion percentages. The percentage figure at the high dose

is significantly different from the other three. While there was not a significant difference between the percentage excretion figures at the 5 and 50 mg/kg doses, the profiles of excretion were different. Prolonged excretion of radiolabel was observed at 50 mg/kg. Also, at 50 mg/kg there was a multipeak excretion profile. Prolonged excretion was also observed, to an enhanced degree, at the 200 mg/kg dose. While the number of ug equivalents excreted in 48 hours at the 200 mg/kg dose was approximately twice the number excreted at 50 mg/kg (tables 30 and 42), the quantities excreted within 17 hours was the same for both doses, namely, 1389 ug equivalents (p. 9). The most logical explanations for this finding would be that there was a saturation of the major metabolic pathway (or of active secretion) for chlorothalonil, or saturation of intestinal absorption, a phenomenon which occurred at a dose somewhere between 5 and 50 mg/kg. Since animals with vastly different amounts of radiolabel in gastrointestinal contents showed essentially no difference in biliary excretion, as reported by the study director (p. 9), it is reasonable to conclude that saturation of biliary secretion accounts for the finding of equal amounts of radiolabel in bile at 17 hours postadministration of either the 50 or 200 mg/kg doses.

3. Urinary Excretion

The percent of the administered dose appearing in urine (combined urine and cage washings) 48 hours postadministration was esentially the same for the 1.5, 5, and 50 mg/kg doses, and averaged 8.08 percent for the three doses combined. For the 200 mg/kg dose, the percent of administered dose appearing in urine was substantially less, 4.73 percent (table 2, p. 33). The study director notes that excretion at the 1.5 and 5 mg/kg doses was rapid. Essentially 94 and 88 percent of the quantities excreted were eliminated within 24 hours. However, for the 50 mg/kg dose, excretion was only 67 percent complete by 24 hours. Excretion as a percentage of dose was still lower at the 200 mg/kg dose. These observations lead to the logical conclusion that, as with biliary excretion, absorption or excretion mechanisms were saturated at the high dose and that saturation probably occurs at a dose somewhere between 5 and 50 mg/kg.

4. Fecal Excretion

Again, as percentages of the administered dose, radiolabel contained in feces 48 hours postadministration was essentially constant for the 1.5, 5, and 50 mg/kg doses, when for the three dose groups combined the mean value for radiolabel excreted in feces was 61 percent. On the average, an additional 3 percent of the administered dose was found in the GI tract, thus 64 percent of the total dose was accounted for in the feces and GI tract at all but the highest dose level. However, at the high dose 32.5 percent was in feces and 26.1 percent in the GI tract, accounting for a combined 58.6 percent of the administered dose. Just why so much more was located in the GI tract at the higher dose is unclear, but may be attributable to, or a reflection of, the high variability among animals at this dose. It was concluded by the study director that no direct relationship existed between fecal elimination, or content of radiation in the GI tract, and biliary excretion (p. 13). This appears to be a reasonable conclusion.

5. Kidney Concentrations

In cannulated rats kidney concentrations of radiolabel 48 hours postadministration of chlorothalonil did not increase in direct proportion to dose. There were progressive deficits with respect to a linear increase with increasing dose, i.e., the response was nonlinear.

When kidney concentration data from cannulated rats obtained at three doses (5, 50, and 200 mg/kg) 48 hours postadministration are included with comparable data from noncannulated rats at the 24-, 96-, and 168-hour time points on a semilog plot of kidney concentration vs. time plot, a continuous linear plot covering 24 to 168 hours is obtained, i.e., data from cannulated rats appears to be superimposable with data from noncannulated rats. This leads to the reasonable conclusion that kidney concentrations were not affected by bile duct cannulation. Hence, enterohepatic circulation or biliary readsorption from the GI tract did not play a significant role in kidney levels (p. 14, Figure 4, p. 38).

6. Recovery of Radioactivity

At the three lower doses, percent recoveries of radiolabel were essentially the same, averaging 92.4 percent. Recovery was significantly less at

the highest dose, namely 74.1 percent. The lower recovery figure for the high dose group may be attributable to the relatively high levels in feces and GI contents and difficulties inherent in measuring such levels in feces and contents.

7. Kinetic Model for Chlorothalonil

A kinetic model is presented which is identical to the model described in the accompanying metabolism study, 1173-84-0079-AM-003, and has been commented upon in the review of the latter study.

Core Rating: Minimum.

005911

Reviewed by: Brian Dementi, Ph.D. Brian Dementi 8/3/87
Section I, Toxicology Branch (TS-769C)
Secondary Reviewer: R. Bruce Jaeger, Section Head
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Metabolism, Rat Tox. Chem. No.: 081901

Accession No.: 264351

Test Material: Mono-Glutathione Conjugate of ^{14}C-Chlorothalonil

Synonyms: ^{14}C-SDS-66382

Study Number: 631-4AM-85-0064-001
Report/SDS-66382

Sponsor: SDS Biotech Corporation
Painesville, OH

Testing Facility: Physical Sciences Laboratories,
SDS Biotech Corp., P.O. Box 348
Painesville, OH

Title of Report: Pilot Study to Determine the Concentration of Radiolabel in Kidneys Following Administration of the Mono-Glutathione Conjugate of ^{14}C-Chlorothalonil to Male Rats

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

Report Issued: April 23, 1986

Purpose of Study:

The purposes of this pilot study appear to have been threefold: 1) to further characterize the route of metabolism of chlorothalonil, 2) ". . . to determine if radiolabel from a dose of a monoglutathione conjugate of chlorothalonil would be found in the kidney in the same relative amount as previously reported for an equimolar dose of chlorothalonil, and 3) to compare oral and intraperitoneal doses of the monoglutathione conjugate with respect to the presence of thiol conjugates of chlorothalonil in the urine" (p. 2).

Conclusions:

The glutathione pathway plays an important role in the metabolism of chlorothalonil as evidenced by the finding

that similar thiol metabolites result whether chlorothalonil or monoglutathione conjugate is administered to the rat. Radiolabeled chlorothalonil and the conjugate yield approximately equivalent percentages of radiolabel in the urine. The oral route of administration results in much higher urinary levels of thiols of chlorothalonil than does the intraperitoneal route, suggesting a role of the gastrointestinal tract in glutathione metabolism.

These are pilot study findings and are only indicative of the involvement of the one metabolic pathway. Additional study further characterizing the metabolism by the glutathione route will be necessary, as well as investigations into the potential role of other pathways.

Critical Review Criteria:

A. Materials:

1. Test Compound: Monoglutathione conjugate of ^{14}C-chlorothalonil.

   Description: The specific activity of the radiolabeled material was 0.576 mCi/mMole. The test material was stored in the dark at -8 °C.

   Purity: The nonlabeled starting material was chlorothalonil of 99.7% purity. The radiochemical purity of the final test material (i.e., the glutathione conjugate) was 91.3%. There was uniform labeling of the benzene ring.

   Contaminants: Not indicated.

2. Test Animals: Species: Rat, male; Strain: CD Sprague-Dawley; Weight: 287 to 332 grams; Source: Charles River Breeding Laboratories, Portage, MI.

B. Study Design:

Testing was performed using a mixture of radiolabeled and nonradiolabeled monoglutathione conjugate of chlorothalonil in a 0.75 percent methylcellulose/water suspension.

"Eight rats were assigned to each of three groups, Group I (oral), Group II (intraperitoneal), and Group III (intraperitoneal pilot). Untreated rats in Group III were used for control tissue.

"Food was removed from the cages of control and experimental rats at approximately 4 P.M. the night prior to dosing. These cages contained a water source but no

food. Just prior to dosing, experimental animals were placed individually in metabolism cages which contained a water source but no source of food. The cages were placed over containers of dry ice to freeze any collected urine. The rats were dosed as close as posible to 8 A.M. Each experimental rat received a single dose of 115 mg SDS-66382/kg in 0.75 percent methylcellulose (115 mg SDS-66382/kg body weight/10 mL suspension). Rats in Group I were dosed orally and those in Group II were dosed intraperitoneally. Control rats (Group III) were not dosed.

"Six hours after administration of SDS-66382, control and experimental rats were sacrificed by exsanguination under ether anesthesia. Kidneys were removed from all animals, and carcasses were stored frozen for future disposal. Prior to termination, blood samples were collected from animals under ether anesthesia by orbital sinus puncture. Blood samples were assayed for radioactivity by combusting aliquots of blood and counting the trapped CO_2 by Liquid Scintillation Counting (LSC).

"Urine samples were collected over dry ice. Cages were rinsed with 50 percent methanol in water to collect any urine which did not flow into the collection cup. The total volume was measured and duplicate 0.1 mL aliquots were assayed for radioactivity by LSC. The urine was stored frozen and subsequently analyzed for sulfhydryl metabolites.

"Kidneys were removed at termination, rinsed twice in a 50 percent methanol/water solution, and then stored in plastic bags. The kidneys were rinsed a third time in 50 percent methanol/water when they were removed from the bags for analysis. These solutions (15 mL each) were subsequently analyzed for radioactivity by LSC. The capsules of the kidneys were removed, and the kidneys were minced with scissors. Aliquots of the kidney tissue were weighed for subsequent biological oxidation and LSC. The remainder of the kidneys were stored frozen. The capsules were analyzed separately for the presence of radiolabel by biological oxidation and subsequent LSC (pp. 11-13)."

Results:

There were no external adverse effects noted for the dosed animals. Necropsy revealed the presence of some fluid (< 1 to 2 mL) in the peritoneal cavities of animals dosed via this route. Via oral administration, the monoglutathione is

much less toxic than chlorothalonil on an equimolar basis, suggesting that glucuronide conjugation of chlorothalonil is probably detoxifying (p. 19).

With respect to blood concentrations of radiolabel it was found that 6 hours postdosing the average blood concentrations were 13.3 mMole-equiv/mL for orally dosed rats and 132.1 mMole-equiv/mL for those dosed intraperitoneally (i.p.) (table 2, p. 27). It is speculated that the evidently rapid absorption by the i.p. route can be attributed to the abundant blood supply and large surface area of the peritoneal cavity.

As was true in the case of blood, levels of radiolabel in the kidney were considerably higher for those rats dosed i.p. (705 nMole-equiv/gram) than for those dosed orally (49.5 nMole-equiv/gram) (table 3, p. 28). The average percentages of the administered doses found in the kidney were 3.22 percent for the i.p. dosed group and 0.20 percent for the group administered orally.

Animals dosed i.p. excreted via the urine much higher percentages of the administered dose than did the orally dosed group. The percentages were 5.35 $\pm$ 4.25 and 0.64 $\pm$ 0.31 percent, respectively (table 4, p. 29).

In a previous study cited by the authors (p. 19), which involved the administration of essentially equimolar doses of radiolabeled chlorothalonil, the percent of the administered dose located in the kidney 6 hours postdosing was close to the percentage found in the present study. Comparisons between percentages of dose found in kidney, blood, and urine for the two studies are tabulated below as taken from the study report (p. 20).

	Oral Chlorothalonil	Oral Chlorothalonil, Monoglutathione	Intraperitoneal Chlorothalonil, Monoglutathione
Kidney (% administered dose)	0.26	0.20	3.22
Blood (% administered dose)	0.24*	0.40	3.96

*This is an estimate, as plasma was assayed rather than whole blood.

	Oral Chloro-thalonil	Oral Chlorothalonil, Monoglutathione	Intraperitoneal Chlorothalonil, Monoglutathione
Urine (% administered dose)	1.19	0.64	5.35
Thiols (% in urine)	8.3	14.1	< 1

Thus, when radiolabeled chlorothalonil or its monoglutathione derivative are administered orally in separate studies at equimolar doses, the percentages of radiolabel located in kidney are very similar, suggesting similar routes of metabolism.

As to the characterization of urinary metabolites resulting from oral dosing with the glutathione derivative, the di- and trithiols of glutathione were identified among other unidentified substances (metabolites). The dithiol derivative accounted for 9 percent of the extractables and the trithiol approximately 5 percent (p. 18). Urine from animals dosed i.p. contained the dithiol (1 percent of extractables) and no trithiol.

Based upon the above limited information, the authors develop the concept that an essential route of metabolism for orally administered chlorothalonil includes glucuronide formation (mono-, di-, and triglucuronides) in the gastrointestinal tract, followed by cleavage to smaller fragments which are absorbed into the portal circulation. The fragments in question are theorized to be cleaved in the kidney to the thiol metabolites (nephrotoxins, p. 23). In support of this, the authors cite a recent in vitro study in which it was shown that mucosal cells from the stomach and small intestine will affect these conjugation reactions. Such reactions in the gastrointestinal tract prior to absorption into the portal circulation would help explain the greater abundance of thiols in the urine following oral dosing as opposed to intraperitoneal dosing.

Evidence in support of this proposed sequence of metabolic events for chlorothalonil include the finding of similar metabolites in urine following dosing with either chlorothalonil or the monoglutathione metabolite. The authors conclude that the glutathione pathway is intimately involved in the metabolism of chlorothalonil. This appears to be a reasonable, but limited, conclusion. Additional study would be necessary to adequately characterize the various aspects of the glutathione and possibly other metabolic pathways.

Core Classification: Minimum.

005911

Reviewed by: Brian Dementi, Ph.D. Brian Dementi, 3/7/87
Section I, Toxicology Branch (TS-769C)
Secondary Reviewer: R. Bruce Jaeger, Section Head 3/23/87
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Metabolism, Rat Tox. Chem. No.: 081901

Accession No.: 264350

Test Material: ^{14}C-Chlorothalonil

Synonyms: ^{14}C-SDS-2787

Study Number: 1173-84-0079-AM-003

Sponsor: SDS Biotech Corporation
Painesville, OH

Testing Facility: Huntingdon Research Centre,
Huntingdon, Cambridgeshire, England
October 19, 1984 to May 1985

Title of Report: Study of the Distribution of Radioactivity Following Repeated Oral Administration of ^{14}C-Chlorothalonil (^{14}C-SDS-2787) to Male Sprague-Dawley Rats

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

Report Issued: July 3, 1986

Special Review Criteria

A. Materials:

1. Test Compound: A mixture of nonlabeled chlorothalonil and ^{14}C-labeled chlorothalonil suspended in 0.75% methylcellulose.

   Description and Purity: The nonlabeled material was analytical grade chlorothalonil of 99.7% purity. ^{14}C-Chlorothalonil was of specific activity 62.4 mCi/mMole having radiochemical purity of 98.4%.

   Batch No.: N/A.

   Contaminants: N/A.

2. Test Animals: Species: Rat, male; Strain: CD Sprague-Dawley; Age: 9 to 10 weeks; Weight: Approximately 300 grams; Source: Charles River Breeding Laboratories, Portage, MI.

B. Study Design:

The purpose of the study is to assess the absorption, tissue distribution, and excretion of radioactivity during 7 days following repeated oral administration of radiolabeled chlorothalonil.

1. Animal Assignment: "Eighty-eight male Sprague-Dawley rats were obtained from Charles River Breeding Labs. Eighty of the rats were allocated at random to four dose groups of 20 animals each and eight rats were allocated to supplemental groups to determine blood concentrations during the dosing regimen. The four groups of 20 animals each were dosed at levels of 1.5, 5, 50, or 160 mg/kg/day for 5 days at 24 hour intervals. Animals were dosed orally using a plastic syringe fitted with an animal feeding needle. The exact weight of the < 2.0 mL volume administered was determined by weighing the syringe before and after compound administration. Four rats at each dose level were terminated 2, 9, 24, 96, and 168 hours after the fifth dose administration. Urine and feces were collected at 24-hour intervals during the dosing regimen, after the fifth dose and at necropsy.

"All eighty animals were killed by exsanguination under halothane/oxygen anesthesia. Liver, kidneys, fat, muscle, heart, lungs, stomach, small and large intestines, stomach contents, small intestinal contents and large intestinal contents were removed from each animal. Blood, tissues and gastrointestinal tract contents were assayed for radioactivity.

"The supplemental group of eight animals was divided into four subgroups of two rats, each representing one dose level. The two rats in each subgroup were dosed on 5 consecutive days at 1.5, 5, 50, or 160 mg/kg/day. Blood samples were collected at 6 and 24 hours after the first, third, and fifth dose administrations and the samples were assayed for radioactivity. The eight rats were sacrificed 24 hours after the final dose in the same manner discussed above but no tissues were collected (pp. 2-3)."

2. Diet Preparation: Animals were fed ad libitum using Spratt's Laboratory Diet No. 1. Animals were also allowed fresh water ad libitum. The test compound was not administered via diet or drinking water, hence no specific diet preparation or water formula was necesary.

3. Statistics: The following procedures were utilized in analyzing the numerical data: N/A.

4. Quality Assurance was affirmed in a statement by J.A. Ignatoski, Ph.D., Director, Department of Safety Assessment, July 3, 1986.

C. Results:

(The following information is paraphrased and follows the outline of results as presented in the study on pages indicated.)

1. Observations: Animals treated at the high dose, 160 mg/kg/day, had loose stools primarily during the 24 hours immediately following the first of five doses in the series. While fecal consistency was essentially normal beyond the initial 24-hour period, many fecal pellets contained a white, mucosal material throughout the study (p. 3).

2. Radioactivity in Feces and Gastrointestinal Tract: Based upon data presented in Appendix A, it is evident that the principal route of elimination of radiolabel is via the feces, where the radiolabel presence accounted for 82 to 85 percent of the total dose administered at the various dose levels. Elimination via this route was rapid and essentially 90 percent complete within 24 hours following the fifth dose administered in all of the various dosing regimens.

   Evacuation time (or rate) of radiolabel from the stomach was dose dependent when evacuation was essentially complete within 9 hours following the final (5th) dose for the 50 mg/kg/day regimen, but only within 24 hours following the 160 mg/kg/day regimen (p. 4).

3. Radioactivity in Urine: The percent of the total radiolabel excreted via urine within 7 days after the fifth dose was dose dependent. For the 1.5 and 5 mg/kg/day doses 6.65 and 6.55 percent, respectively, were eliminated, whereas for the

50 and 160 mg/kg/day doses 4.36 and 4.96 percent were eliminated under like circumstances. The average amount of radiolabel excreted in urine during 24 hours following each dose was a constant for each dose level. The data suggest a different mechanism of elimination via the urine for the low dose as opposed to the high dose groups (pp. 4-6).

4. Radioactivity in Blood: Data for all dose levels reveal that less than 1 percent of any dose level administered was present in the blood at any moment of sampling. Peak blood concentrations at the various doses occurred at the indicated time points after the final dose.

Dose, mg/kg/day	Peak Blood Concentration, Hours	Blood Level, ng equiv/mL
1.5	2	185
5	2	519
50	9	4300
160	2, 9	12,950

At all dose levels, blood concentrations at the 6-hour time point after the first dose were equivalent to those concentrations evident 6 hours after the final dose, suggesting to this reviewer that a steady state or equilibrium is established quickly and is well maintained (pp. 6-7).

5. Radioactivity in Kidneys: When assayed for radiolabel at various time points during 2 to 168 hours after the fifth dose, the highest concentrations in kidneys occurred at the 2-hour time point, regardless of dose level. These peak concentrations were 3.12, 8.03, 31.1, and 105 ug equiv/g at the respective doses of 1.5, 5, 50, and 160 mg/kg/day.

Kidney concentrations were proportional to dose for the two lower doses (~ 0.1 percent of administered dose/g) and also for the two higher doses (~ 0.05 percent of administered dose/g), but proportionalities did not hold between the second and third dose groups.

Plots of kidney depletion rates obtained for 1.5, 5, and 160 mg/kg/day dose groups indicate depletion is biphasic, with the phase change occurring at 24 hours after the final dose. For the 50 mg/kg/day dose, elimination was not biphasic (pp. 7-9).

Dose, mg/kg/day	Rate Constant, Hour^{-1}		
	2-24 hrs	24-168 hrs	2-168 hrs
1.5	.0369	.0078	
5			.0091
150	.0298	.0078	
160	.0300	.0047	

We are unable to offer a reliable explanation for the single rate constant observed at the 5 mg/kg/day dose.

6. Radioactivity in Other Tissues: Concentrations of radiolabel in fat, heart, liver, lungs, and muscle were unremarkable. GI tissues, apart from contents containing radiolabel, also appeared unremarkable. Kidney tissue had the highest radiolabel concentration and was five- to sevenfold that of the liver, the tissue incorporating the next highest concentration of radiolabel. Radiolabel depletion rate for liver was threefold that of the kidneys (p. 9).

D. Chlorothalonil Kinetics Model:

The petitioner has developed a theoretical model for chlorothalonil kinetics. This model is based upon the assumption that ". . . chlorothalonil absorption and excretion may be described by a one-component model, where chlorothalonil and/or its metabolites are absorbed into the bloodstream and eliminated from the blood compartment by distribution to tissues, by excretion into bile and by excretion into urine" (p. 10). The mathematical expression of this model as derived would be:

$$V_A = V_T + V_B + V_U$$

where

V_A = rate of absorption into blood;
V_T = rate of absorption into tissues from blood;
V_B = rate of elimination via bile; and
V_U = rate of elimination via urine.

This is a simple model not taking into consideration a number of factors which could influence rates of distribution. Nevertheless, when values for V_T, V_B, and V_U, arrived at by deductive application of more fundamental information pertaining to these three parameters as obtained in ancillary studies, are introduced into the

model equation, values for V_A can be calculated or estimated at various doses of chlorothalonil. For example, employing this technique, the petitioner obtains V_A = 143.63 ug/equiv/hr at a dose of 50 mg/kg and 258.41 ug/equiv/hr at the 200 mg/kg dose. The ratio of these, 258.41/143.63 = 1.8, indicates that the rate of absorption nearly doubles when the dose increases fourfold, within the indicated dose range.

In attempting to establish the validity of the model equation, the petitioner also employs actual biliary and urinary excretion data obtained in the adjunct biliary study (633-4AM-85-0012-002) to determine actual amounts and ratios of chlorothalonil absorbed within 24- and 48-hour time periods at doses of 50 and 200 mg/kg. Thus within 24 hours post-administration of 50 mg/kg and 200 mg/kg, 2780.8 ug and 5389.8 ug equivalent were absorbed, the ratio being 1.94.

The same ratio calculated in like manner at 48 hours was 2.12 (pp. 10-19). Both ratios (1.94 and 2.12) compare favorably with the 1.8 ratio derived from more fundamental principles. Hence, the petitioner considers these findings to support the one-component model as proposed to describe the kinetics of chlorothalonil absorption and excretion. To a first approximation, this appears to be reasonable.

Conclusions:

1. The relative rate of absorption of chlorothalonil following a 200 mg/kg dose is only approximately twice that following administration of a 50 mg/kg dose.

2. Less than 1 percent of any single dose of chlorothalonil administered is present in the blood at any moment. Peak blood concentrations following single doses were approximately dose related. An apparent saturation of blood occurs during multiple dosing between the dosing rates of 5 and 50 mg/kg/day and is indicative of a steady state.

3. Radiolabel depletion from kidney was biphasic at doses of 1.5, 5, and 160 mg/kg/day, but not so at 50 mg/kg/day. There is no satisfactory explanation for this difference.

4. The data suggest a different mechanism of elimination via the urine for the two low dose groups as opposed to the two high dose groups.

5. The principal route of elimination of radiolabel is fecal, accounting for 82 to 85 percent of administered dose. Elimination via this route was rapid and essentially 90 percent complete within 24 hours following the final dose. These findings were independent of dose level under study and indicate most chlorothalonil is excreted unchanged in the feces. Furthermore, this is consistent with the low percentage of the administered dose present in the blood at any moment post-administration.

6. A theoretical model for chlorothalonil kinetics ($V_A = V_T + V_B + V_U$) was proposed where the rate of absorption into the blood (V_A) is equivalent to the sum of the rates of transfer into tissues (V_T) and elimination via bile (V_B) and urine (V_U). To a first approximation the model appears reasonable.

Core Rating: Minimum.