DATA EVALUATION RECORD

CHLOROTHALONIL

Study Type: §85-1; Metabolism Study in Rats

Work Assignment No. 3-01-91 D (MRID 45710216)

Prepared for
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Building 100, Suite B
Durham, NC 27713

Primary Reviewer:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 11/11/05

Secondary Reviewer:
John W. Allran, M.S.

Signature: John W. Allran
Date: 11/11/05

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 11/14/05

Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T.

Signature: Steven Brecher
Date: 11/14/05

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: William Greear, MPH, D.A.B.T. **Signature:** William Greear
Registration Action Branch 1, Health Effects Division (7509C) **Date** 12/14/2005
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date** 12/15/05

TXR#: 0052493

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat; OPPTS 870.7485 (§85-1); OECD 417.

PC CODE: 081901 **DP BARCODE:** D301496

TEST MATERIAL (RADIOCHEMICAL PURITY): [Phenyl-U-^{14}C]-Chlorothalonil (>97%)

SYNONYM: 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile; tetrachloroisophthalonitrile;

CITATION: Aikens, P.J. (1997) Chlorothalonil: metabolism in the rat. Huntingdon Life Sciences, Ltd., Huntingdon, Cambridgeshire, England. Laboratory Study ID: VCM 66/963515, April 25, 1997. MRID 45710216. Unpublished.

SPONSOR: Vischim S.r.l., Via Friuli, Cesano Maderno, Milan, Italy

EXECUTIVE SUMMARY: In a rat metabolism study (MRID 45710216), [Phenyl-U-^{14}C]-Chlorothalonil (Batch # 1021; radiochemical purity >97%) in 1.0% (w/v) aqueous sodium carboxymethylcellulose was administered to Sprague-Dawley (Crl:CD®BR) rats by gavage. A single dose of the compound was administered at 1.5 or 50 mg/kg nominal in the following studies: excretion balance/tissue distribution (five rats/sex/dose); plasma pharmacokinetics (ten rats/sex/dose); biliary excretion (three males/dose); enterohepatic recirculation (three males treated with donor bile from a 1.5 mg/kg male); and tissue metabolite profile (three/sex treated with 1.5 mg/kg only). Metabolites were quantified and identified in urine and feces from the excretion balance study, bile from the biliary excretion and enterohepatic recirculation studies, and plasma, liver, and kidney from the tissue metabolite profile study.

After 120 h, total recoveries ranged from 91.6-101% of the administered doses, with no differences observed between doses or sexes. The test compound was rapidly absorbed, as radioactivity was detected in plasma at 0.25 h (first time point analyzed). At the low dose, maximum plasma concentrations were observed after two to four h, increasing to 12 h at the high dose. Differences in the increase in administered dose compared to increases in the maximum plasma concentration and area under the curve, along with the observed increase in the time to maximum plasma concentration suggested that dose-dependent kinetics occurred for both the rate and extent of absorption of the test compound in both male and female rats. A pilot excretion study indicated that $^{14}CO_2$ was not exhaled during the first 48 h post-dosing. The majority of the radioactivity was recovered in the feces; males appeared to excrete a greater proportion in the first 24 h, while females excreted closer proportions during 0-24 h and 24-48 h. Approximately one-half of the administered dose was extractable from the feces. Urine accounted for a smaller

percentage of the dose, with females appearing to excrete a greater proportion of radioactivity than males. The majority of radioactivity was excreted in the urine and feces within 48 h post-dosing. Cage wash, combined tissues, and carcass accounted for <=0.36% of the administered dose. Bile also was a major excretion pathway, accounting for 8.7-18.4% of the administered dose by 48 h, with urine and fecal excretion profiles similar to those observed in non-bile duct-cannulated animals. Approximately 23% of the administered dose was absorbed by the low dose males, while only approximately 14% of the administered dose was absorbed by the 50 mg/kg males. Animals dosed with donor bile directly into the duodenum demonstrated radioactivity in the urine and bile, indicating enterohepatic recirculation was taking place.

The test compound was not extensively retained by any tissue. At 120 h post-dose, radioactivity was detected in the kidneys at both doses, with females retaining more radioactivity than males at both the low (0.144 vs 0.077 µg Eq/g) and high (4.42 vs 2.27 µg Eq/g) doses. High dose females demonstrated greater overall retention and distribution of radioactivity. Whole blood and plasma demonstrated approximately equal amounts of radioactivity, indicating radioactivity was not partitioned into red blood cells. In all other tissues, the concentration of radioactivity was less than plasma levels or below the limit of accurate detection.

HPLC analysis of urine, bile, and feces revealed broadly similar patterns of metabolites, with up to 18 distinct peaks resolved. Parent was present in the fecal extracts at 4.3-7.5% of the administered dose in the 1.5 mg/kg group, and at 19.8-23.9% in the 50 mg/kg group. Parent was not present in the urine or bile. No fraction accounted for >5% of the administered dose in urine or bile, with the exception of fraction 6, which accounted for 5.0% of the administered dose in bile from the enterohepatic recirculation study. Fraction 6 was observed in urine, feces, and bile, and was confirmed as the monoglutathione conjugate of Chlorothalonil by TLC co-chromatography. Fractions 7, 12, and 13, generally present in urine, feces, and bile, were identified by LC/MS as mercapturic acid conjugates. Analysis following treatment of urine and bile with β-glucuronidase/sulphatase showed no evidence of deconjugation. It was stated that a component representing up to 4.8% of the dose in fecal extracts was not retained by HPLC and could not be further separated or characterized.

This metabolism study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OPP 85-1] in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP compliance, and Quality Assurance statements were provided.

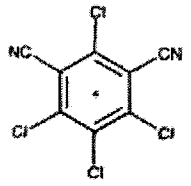
I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound:

Radiolabeled test material 1: [Phenyl-U-^{14}C]-Chlorothalonil

Radiochemical purity:	>97% (determined by TLC)
Specific Activity:	47.5 mCi/mmol (178.6 µCi/mg)
Batch No.:	1021
Structure:	



(po sition of ^{14}C-label indicated by *)

Non-radiolabeled test material 1: Chlorothalonil

Description:	Not provided
Batch No.:	14/09/93/1
Purity:	99.8%
CAS # of TGAI:	1897-45-6

Non-radiolabeled test material 2:

Batch No.:	Q037
Purity:	99.5%

Non-radiolabeled test material 3:

Batch No.:	25
Purity:	99.18%

2. Vehicle: 1.0% (w/v) aqueous sodium carboxymethylcellulose solution

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD®BR)
Age and weight at dosing:	6-10 weeks 201-224 g males; 208-224 g females
Source:	Charles River UK Ltd. (Margate, Kent, UK)
Housing:	In excretion balance experiments, rats were individually housed in glass metabolism cages; during biliary excretion studies, rats were housed in restraining cages; during acclimatization and blood/plasma pharmacokinetic and metabolite determination experiments, rats were housed in stainless steel cages with suspended mesh floors

Diet:	LAD I pellets (SDS Witham, Essex, UK), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	22±2°C
	Humidity:	40-60%
	Air changes:	Approximately 15/h
	Photoperiod:	12 h light/dark
Acclimation period:	At least 5 days	

4. Preparation of dosing solutions: A stock solution was prepared by dissolving the radiolabeled test substance in acetonitrile. Measured volumes of this stock solution were mixed with non-radioactive Chlorothalonil to make two stock formulations that were used for the preparation of the low (1.5 mg/kg) and high (50 mg/kg) level doses. The specific activities of these stock formulations were 33 and 1.6 µCi/mg (determined by HPLC) for the low and high level solutions, respectively. To make the dosing solutions, an appropriate volume of stock formulation was concentrated to dryness under nitrogen, and then resuspended in a calibrated volume of 1.0% (w/v) aqueous carboxymethylcellulose solution using a drill-mounted Teflon pestle. Dosing solutions were prepared at nominal concentrations of 0.3 or 10 mg/mL.

For the enterohepatic recirculation experiment, bile was collected from a donor animal given the 1.5 mg/kg dose formulation. Triplicate aliquots (determined by weight) were analyzed to measure the concentration of radioactivity in the bile sample and the amount of radioactivity administered to each animal.

No information was provided concerning the stability of the preparations. It was not stated if the solutions were stirred continuously following preparation to maintain suspension; however, it was stated that doses were administered within two h of preparation. Triplicate aliquots of the prepared dosing solutions were analyzed by liquid scintillation counting (LSC) to confirm homogeneity and concentration. The radiochemical purity of the dosing solutions were 97.5-99.1% (determined by TLC), and the specific activities of the formulations were 73,249 dpm/µg for the 1.5 mg/kg dose groups and 3546 dpm/µg for the 50 mg/kg dose groups.

B. STUDY DESIGN AND METHODS

1. Group arrangements: Animals were assigned to the test groups presented in Table 1. It was stated that the nominal dose levels were selected based on results from previously performed toxicology studies. Additionally, prior to the initiation of the metabolism studies, two rats/sex were administered non-radioactive test compound at 50 mg/kg and observed for clinical signs of toxicity; it was stated that no effects were observed at 24 h post-dosing. Animals were weighed on the day of dosing.

Table 1. Single dose groups for [^{14}C]-Chlorothalonil metabolism study[a]

Nominal dose (mg/kg)	Mean actual dose (mg/kg)	# animals/ group	Comments
Pilot Excretion Studies			
1.5	2.5	1/sex	Feces were collected daily for five days; urine was collected at 0-6 and 6-24 h, then daily for five days. Expired air was monitored daily for two days. A cage wash was also collected. On Day 5, rats were killed by cervical dislocation and the carcasses retained.
50	42.0 male 42.3 female		
Excretion Balance/Tissue Distribution Studies			
1.5	1.2	5/sex	Feces were collected daily for five days; urine was collected at 0-6 and 6-24 h, then daily for five days. A cage wash was also collected. On Day 5, rats were killed by cervical dislocation; blood and selected organs were collected for radioactivity analysis.
50	45.0 males 44.9 females		
Plasma Pharmacokinetic Studies			
1.5	1.2	10/sex	Blood samples were collected from a tail vein pre-dose, and at regular intervals post-dosing. Blood samples were centrifuged and the plasma was taken for radioactive analysis.
50	43.1 males 43.8 females		
Biliary Excretion Studies			
1.5	1.5	3 males	Bile samples were collected at regular intervals. Urine was collected at 0-6, 6-12, 12-24, and 24-48 h; feces were collected every 24 h. At 48 h, rats were killed by cervical dislocation, and the carcass was retained. A cage wash was also collected.
50	57.0		
Enterohepatic Recirculation Study			
1.5	2.5	3 males	A donor animal was dosed and bile samples collected at regular intervals. Animals were then dosed with bile from the donor animal, and samples were collected as in the biliary excretion studies.
Tissue Metabolite Profile Study			
1.5	1.2	3/sex	Animals were killed at the time of peak plasma concentration, and blood, kidneys, and liver were collected for metabolite analysis. Pooled urine and fecal samples from the excretion balance studies, and bile from the biliary excretion and enterohepatic recirculation studies were analyzed for metabolites. Samples were analyzed/purified by TLC, HPLC, and/or LC/MS.

a Data were obtained from pages 20-22, 30-31, and Appendix 1 on pages 81-85 of the study report.

2. Dosing and sample collection: Animals were dosed by oral gavage with dose amounts based on individual body weights. Dose volumes of 1.0 or 1.1 mL were administered; however, no information was presented concerning how the actual amount administered was determined. It was stated that the doses of bile administered to animals in the enterohepatic recirculation study were determined gravimetrically. It was not stated whether all animals were fasted prior to dosing. The daily doses administered approximated the nominal doses. The actual mean administered doses and the number of animals treated in each study are reported in Table 1.

a. Pharmacokinetic studies: All studies were performed with single oral doses of the test compound except the biliary excretion and enterohepatic recirculation studies described below.

i. Pilot excretion studies: Rats were dosed with either 1.5 or 50 mg/kg of the radiolabeled test compound and housed in metabolism cages. Feces were collected daily for five days; urine was collected in dry ice-cooled receivers at 0-6 and 6-24 h, and then daily for five days. Expired air was monitored over a two-day period at daily intervals by passing the expired air over two CO_2 traps containing 3 : 1 (v/v) 2-ethoxyethanol:ethanolamine. An aqueous cage wash was collected and analyzed separately. Radioactivity was determined in urine, feces, cage wash, expired air, and carcass.

ii. Excretion balance/tissue distribution studies: Rats were dosed with either 1.5 or 50 mg/kg of the radiolabeled test compound and housed in metabolism cages. Urine samples were collected in dry ice-cooled receivers at 0-6 and 6-24 h and then at daily intervals up to five days post-dosing. Feces were collected at daily intervals for up to five days post-dosing. On Day 5, a blood sample was taken, the animals were killed by cervical dislocation, and the following tissues were collected:

GI tract (and contents)	Heart	Lung	Spleen	Thyroid	Skeletal muscle	Bone marrow
Adrenals	Kidneys	Ovaries	Testes	Parathyroids	Abdominal fat	Carcass
Brain	Liver	Pancreas	Thymus	Uterus	Bone	

Radioactivity was determined in urine, feces, cage wash, tissues, blood, plasma, and residual carcass.

iii. Plasma pharmacokinetic studies: Rats were dosed with either 1.5 or 50 mg/kg of the radiolabeled test compound, and then were divided into two groups of five rats/sex/dose. Blood samples were drawn from a tail vein from the groups at the following time points:

Group 1: pre-dose, 0.5, 2, 6, 24, and 72 h
Group 2: 0.25, 1, 4, 12, 48, and 96 h.

Additionally, samples were taken at 120 h from the low dose Group 1 animals only. The samples were centrifuged and the plasma analyzed for radioactivity.

iv. Biliary excretion study: The bile ducts and stomachs of all rats were cannulated under anesthesia. Animals were allowed to recover from the anesthesia, and doses of either 1.5 or 50 mg/kg of the radiolabeled test compound were administered via the stomach cannula. After dosing, a 28 mg/mL solution of taurocholic acid in aqueous saline was infused via the stomach cannula at a rate of 0.9 mL/h to ensure adequate production of bile. Bile samples were collected deep frozen at 0-2, 2-4, 4-6, 6-12, 12-24, and 24-48 h post-dosing. Urine was collected at 0-6, 6-12, 12-24, and 24-48 h; feces were collected at 24 and 48 h post-dose. Animals were killed by cervical dislocation, and the residual carcass was retained. Radioactivity was determined in urine, feces, bile, cage wash, GI tract, and carcasses.

v. **Enterohepatic recirculation study:** One donor male rat was given a single oral dose of 1.5 mg/kg of the radiolabeled test compound, and then was cannulated as described in the biliary excretion studies. Bile was collected deep frozen at the same time points as described above. The bile from 2-12 h was pooled and administered via the stomach cannula to three additional male rats. Samples were collected as described in the biliary excretion studies.

vi. **Tissue metabolite profile studies:** Rats were dosed with 1.5 mg/kg of the radiolabeled test compound, and were killed at the time of peak plasma concentration for each sex. Blood, kidneys, and liver were collected from these animals for radioanalysis. Additionally, pooled urine and feces from the excretion balance studies, and pooled bile from the biliary excretion studies were analyzed for metabolite content.

vii. **Sample preparation and analysis:** Fecal samples from the 0-24 h interval of the excretion balance and biliary excretion studies were homogenized in ethyl acetate:acetonitrile (1:1 v/v), centrifuged, and then further extracted with ethyl acetate:acetonitrile, acetonitrile, and acetonitrile:water (1:1 v/v). Fecal samples from the 24-48 h interval of the biliary excretion experiment were extracted five times with the procedure and solvent sequence described previously. Fecal samples from the 24-48 h interval of the excretion balance studies and all fecal samples from the enterohepatic recirculation study were extracted once with acetonitrile:water. After extraction, the residues were either dried or homogenized with water to a smooth paste; fecal samples that were not extracted were homogenized with water. Fecal extracts, residues and unextracted fecal homogenates were analyzed for radioactivity. The adrenals, bone marrow, ovaries, and thyroid were solubilized directly with NCS-II solubilizer; other tissues (brain, fat, heart, kidneys, liver, lungs, muscle, pancreas, spleen, testes, thymus, and uterus) were first homogenized with scissors prior to solubilization. The GI tract and contents were separated into four compartments (stomach, intestinal tract, and stomach and intestinal contents) and homogenized. Carcasses were digested overnight in a mixture of water, methanol, Triton X-405, and sodium hydroxide at 55°C, and samples were neutralized with nitric acid prior to radioassay.

Radioactivity was measured by liquid scintillation counting (LSC). Liquid samples (urine, bile, cage washes, plasma, tissue and fecal extracts, expired air traps, and carcass digests) were mixed with scintillant and counted. Samples of fecal residues, bone, GI tract and contents, liver, lungs, spleen, and whole blood were combusted in oxygen, trapped, mixed with scintillant and counted. It was stated that radioactivity in amounts less than twice background levels was considered to be below the limit of accurate measurement.

b. **Metabolite characterization:** The urine, bile, fecal extracts, plasma, liver, and kidney were analyzed by HPLC, TLC, and/or LC/MS to determine the metabolite profiles.

Urine samples from the excretion balance studies collected during 0-24 h were pooled separately according to sex and dose level. Pools were analyzed by HPLC and TLC; selected pools were co-chromatographed with selected reference standards. Samples from the low dose females and high dose males were acidified to pH 5.0 with sodium acetate buffer. β-Glucuronidase/sulfatase was added to an aliquot of the acidified urine, and both acidified and enzyme-treated urine were incubated overnight at 37°C. At the end of the incubation period, the enzyme-treated sample was tested to ensure the enzyme was still active. Aliquots of treated urine were analyzed by HPLC.

Additionally, a sample from the high dose females was acidified to pH 2.0, extracted with ethyl acetate, concentrated to dryness, resuspended in water, fractionated by HPLC, and then the isolated metabolites were analyzed by LC/MS.

Samples of bile from the biliary excretion and enterohepatic recirculation studies collected during 0-48 h were pooled separately and analyzed by HPLC and TLC. Selected pools were co-chromatographed with selected reference standards. A sample from the low dose bile pool also was acidified, treated with enzyme, and analyzed as described above. Additionally, an aliquot of the high dose bile pool was acidified, extracted with ethyl acetate, concentrated to dryness, resuspended in water, and analyzed by HPLC.

Fecal extracts collected during 0-24 and 24-48 h were pooled separately according to collection period, sex, and dose level. Samples of the pools from the 24-48 h interval were analyzed directly. Samples of the 0-24 h extracts were evaporated to dryness and redesolved in acetonitrile. Samples were analyzed by HPLC and TLC; selected samples were co-chromatographed with reference standards.

Plasma pools from the tissue metabolite profile study were extracted twice with acetone, concentrated to dryness, resuspended in acetonitrile:water, and analyzed by HPLC. Co-chromatography was performed with selected reference standards.

Liver and kidney pools from the tissue metabolite profile study were subsampled and extracted three times with acetonitrile:ethyl acetate (1:1 v/v) followed by three extractions with acetonitrile:water (1:1 v/v). The residue following all extractions was dried, weighed, and portions combusted to assess recovery. The extracts were pooled, concentrated, and analyzed by HPLC; co-chromatography with selected reference standards also was performed.

3. **Statistics:** Statistical analyses were limited to calculations of mean and standard deviation.

II. RESULTS

A. PHARMACOKINETIC STUDIES

1. **Pilot excretion studies:** Total recovery of the administered radioactive doses in the preliminary study at 120 h post-dose was 78.0-82.2% in the low dose animals and 88.2-91.7% in the high dose animals. In both sexes, the majority of the dose was recovered within 48 h in the feces (73.2-83.0%) and urine (3.9-6.8%). By 120 h post-dose, fecal excretion accounted for 73.9-84.6%, urinary excretion accounted for 4.0-6.9%, and the cage wash accounted for ≤0.02%. $^{14}CO_2$ was not detected in exhaled air; therefore, expired air traps were not used in the excretion balance studies.

2. **Excretion balance/tissue distribution studies:** After 120 h, total recoveries (Table 2a) ranged from 91.6-101% of the administered doses, with no differences observed between doses or sexes. The majority of the radioactivity was recovered in the feces (82.4-92.7% of the administered dose); males appeared to excrete a greater proportion in the first 24 h (75.7-77.5%),

while females excreted closer proportions during 0-24 h (44.1-49.9%) and 24-48 h (34.8% for both doses). Approximately 29.7-40.2% of the administered dose was extracted from the feces collected from 0-48 h in the low dose animals, while 48.1-57.2% was extracted from the high dose group. Urine accounted for a smaller percentage of the dose (4.7-9.0%), with females appearing to excrete a greater proportion (8.3-9.0%) compared to males (4.7-5.6%). The majority of radioactivity was accounted for in both excreta during the first 48 h. Cage wash accounted for 0.03-0.08% of the dose, while tissues and carcass accounted for ≤0.36% of the administered dose.

Table 2a. Mean (% administered ± sd) dose of radioactivity following a single oral dose of [^{14}C]-Chlorothalonil[a]

Matrix	1.5 mg/kg		50 mg/kg	
	Male	Female	Male	Female
Urine 0-6 h	3.51±1.00	3.48±1.86	1.93±0.52	1.64±0.50
6-24 h	1.73±0.46	5.30±2.68	2.47±0.51	5.15±1.26
24-48 h	0.20±0.13	0.73±0.45	0.15±0.07	1.59±0.56
48-120 h	0.11	0.17	0.10	0.27
Total urine	5.55±1.20	8.98±1.06	4.65±0.24	8.32±1.16
Feces 0-24 h	75.7±10.2	44.1±13.5	77.5±8.5	49.9±19.16
24-48 h	14.1±8.7	34.8±12.2	12.5±7.7	34.8±17.9
48-120 h	1.83	3.53	2.68	7.38
Total feces	91.7±4.3	82.4±4.7	92.7±2.2	92.1±1.4
Cage wash	0.03±0.02	0.06±0.02	0.07±0.05	0.08±0.04
Tissues	0.13±0.05	0.16±0.03	0.07±0.02	0.14±0.01
Carcass	0.19	<0.18	0.11	0.22±0.06
Total recovery	97.4±4.0	91.6±4.8	97.6±2.1	101±0.9

a Data were obtained from Tables 7-8 on pages 49-50 of the study report; data are the mean of five rats/sex/group.

The test compound was not extensively retained by any tissue. At 120 h post-dose (Table 2b), radioactivity was detected in the kidneys at both doses, with females retaining more radioactivity than males at both the low (0.144 vs 0.077 µg Eq/g) and high (4.42 vs 2.27 µg Eq/g) doses. Radioactivity was also detected in the thyroid of the high dose females (0.65 µg Eq/g), but was below the limit of detection in the high dose males and at the low dose. High dose females demonstrated greater overall retention and distribution of radioactivity. Whole blood and plasma demonstrated approximately equal amounts of radioactivity, indicating radioactivity was not partitioned into red blood cells. In all other tissues (excluding low residual levels in the stomach of the low and high dose males), the concentration of radioactivity was less than plasma levels or below the limit of accurate detection.

Table 2b. Mean concentrations of radioactivity (µg equivalents/g ± sd) in selected tissues of rats at 120 h following a single oral dose of [^{14}C]-Chlorothalonil[a]

Matrix	1.5 mg/kg		50 mg/kg	
	Male	Female	Male	Female
Kidneys	0.077±0.015	0.144±0.039	2.27±0.338	4.42±0.606
Thyroid	ND	ND	ND	0.653±0.201
Plasma	0.005±0.001	0.010±0.001	0.097±0.037	0.375±0.075
Whole blood	0.004±0.001	0.008±0.001	0.098±0.031	0.352±0.060

a Data were obtained from Tables 12-13 on pages 54-55 of study report; data are the mean of five rats/sex/group.
ND Below the limit of detection

3. **Plasma pharmacokinetic studies:** The test compound was rapidly absorbed, as radioactivity was detected in plasma at 0.25 h (first time point analyzed). At 1.5 mg/kg, a mean maximum plasma concentration (C_{max}) of 0.23 µg equivalents/mL was observed after two h (T_{max}) in the males; a C_{max} of 0.19 µg equivalents/mL was observed after four h in the females(Table 3). The AUC_{96-120} was 3.3 µg equivalents•h/mL for the males and 2.9 µg equivalents•h/mL for the females. The half life ($t_{½}$) was longer in females (57.2 h) than in the males (44.1 h). At 50 mg/kg, C_{max} was much greater in the females (9.3 µg equivalents/mL) than in the males (2.8 µg equivalents/mL), both occurring at a T_{max} of 12 h. Similarly, the AUC_{96-120} was 168.3 µg equivalents•h/mL in the females compared to 66.5 µg equivalents•h/mL in the males. The $t_{½}$ could not be calculated for the females at this dose. Differences in the increase in administered dose compared to increases in C_{max} and AUC, along with the observed increase in T_{max} suggested that dose-dependent kinetics occurred for both the rate and extent of absorption of the test compound in both male and female rats.

Table 3. Mean plasma pharmacokinetic parameters for radioactivity following a single oral dose of [^{14}C]-Chlorothalonil[a]

Parameter	1.5 mg/kg		50 mg/kg	
	Male	Female	Male	Female
Actual dose (mg/kg)	1.2	1.2	43.1	43.8
C_{max} (µg equivalents/mL)	0.227	0.189	2.8	9.3
T_{max} (h)	2.0	4.0	12.0	12.0
AUC_{96-120} (µg equivalents•h/mL)	3.3	2.9	66.5	168.3
AUC (µg equivalents•h/mL)	3.8	3.7	75.0	NC
k_{el} (h^{-1})	0.0157	0.0121	0.0174	NC
$t_{½}$ (h)	44.1	57.2	39.9	NC

a Data were obtained from Table 4 on page 46 of the study report; data are the mean of five rats/sex/group.
NC Parameter could not be calculated with the available data

4. **Biliary excretion:** Bile was a major excretion pathway (Table 4). In bile duct-cannulated males, total recovery of the administered dose was 93.4-104%, with 8.7-18.4% of the administered dose recovered in the bile. Urine accounted for 4.4-5.5% of the administered dose, while feces accounted for 63.7-85.9% of the dose. The carcass accounted for 3.5-5.2%, and minor amounts of radioactivity (≤1.8%) were found in the GI tract and the cage wash. From these data, it can be calculated that approximately 23% of the administered dose was absorbed by

the 1.5 mg/kg males, while only approximately 14% of the administered dose was absorbed by the 50 mg/kg males.

Table 4. Mean recovery (% of administered dose ± sd) of radioactivity from bile duct-cannulated male rats following a single oral dose of [^{14}C]-Chlorothalonil[a]

Matrix	1.5 mg/kg	50 mg/kg
Urine 0-24 h	3.95	4.32
24-48 h	0.49±0.13	1.13±0.65
Total urine	4.44±0.47	5.46±0.83
Feces 0-24 h	29.2	43.8
24-48 h	40.6±22.8	42.7±6.19
Total feces	63.7±3.07	85.9±9.83
Bile 0-24 h	17.20	7.12
24-48 h	1.22±0.84	1.60±0.71
Total bile	18.4±3.11	8.73±1.50
Gastrointestinal tract	1.78±0.93	ND
Carcass	5.18±7.90	3.47±3.05
Cage wash	0.31±0.15	0.21±0.18
Total	**93.4±7.99**	**104±6.37**

a Data obtained from Table 11 on page 53 of the study report; data are the mean of three rats/sex/group.
ND Below the limit of detection

5. <u>Enterohepatic recirculation studies</u>: Animals dosed with donor bile directly into the duodenum demonstrated radioactivity in the urine and bile, indicating enterohepatic recirculation was taking place (Table 5). In these males, total recovery of the administered dose was 108%, with 26.5% of the administered dose recovered in the bile. Urine accounted for 6.2% of the administered dose, while feces accounted for 74.6% of the dose. The carcass accounted for <15.1%, and minor amounts of radioactivity (<1.1%) were found in the GI tract and the cage wash.

Table 5. Mean recovery (% of administered dose ± sd) of radioactivity from bile duct-cannulated male rats following duodenal administration of donor bile from a rat given a single 1.5 mg/kg oral dose of [^{14}C]-Chlorothalonil[a]

Matrix	Donor bile
Urine 0-24 h	5.40
24-48 h	1.30±1.33
Total urine	6.24±4.95
Feces 0-24 h	52.6
24-48 h	22.0±1.76
Total feces	74.6±5.90
Bile 0-24 h	25.96
24-48 h	0.60±0.34
Total bile	26.5±2.75
Gastrointestinal tract	<1.1
Carcass	<15.1
Cage wash	0.85±0.24
Total	**108±6.56**

a Data obtained from Table 11 on page 53 of the study report; data are the mean of three rats/sex/group

B. METABOLITE CHARACTERIZATION STUDIES: HPLC analysis of urine, bile, and feces revealed broadly similar patterns of metabolites, with up to 18 fractions resolved. Parent was present in the fecal extracts at 4.3-7.5% of the administered dose in the 1.5 mg/kg group, and at 19.8-23.9% in the 50 mg/kg group. Parent was not present in the urine or bile. No fraction accounted for >5% of the administered dose in urine or bile, with the exception of fraction 6, which accounted for 5.0% of the administered dose in bile from the enterohepatic recirculation study. Fraction 6 was observed in urine, feces, and bile, and was confirmed as the monoglutathione conjugate of Chlorothalonil by TLC co-chromatography. Fractions 7, 12, and 13, generally present in urine, feces, and bile, were identified by LC/MS as mercapturic acid conjugates. Analysis following treatment of urine and bile with β-glucuronidase/sulphatase showed no evidence of deconjugation. It was stated that a component representing up to 4.8% of the dose in fecal extracts was not retained by HPLC and could not be further separated or characterized.

Pools of liver, kidney, and plasma were extracted and analyzed by HPLC. The extracts represented 50.0-59.3% of the liver radioactivity, 39.1-47.7% of the kidney radioactivity, and 72.7-78.1% of the plasma radioactivity. Fraction profiles were generally similar across all groups.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The pattern of excretion of [^{14}C]-Chlorothalonil in intact rats was broadly similar at 1.5 and 50 mg/kg dose levels. Radioactivity was excreted via the urine and feces. Female rats excreted a higher proportion of radioactivity in the urine. Absorption of radioactivity was generally low. After five days, low levels of radioactivity were detected in the tissues of male animals, except the kidney. Radioactivity levels were generally higher in the tissues of females, and more extensive tissue distribution was detected. A large number of metabolites were seen in urine and bile. Metabolites seen in both urine and bile included the monoglutathione conjugate of Chlorothalonil, and species containing mercapturic acid moieties. The highest proportion of radioactivity was excreted in the feces. Radioactivity extracted from the matrix consisted primarily of parent, and a large proportion of fecal radioactivity remained bound in the fecal matrix.

B. REVIEWER COMMENTS: After 120 h, total recoveries ranged from 91.6-101% of the administered doses, with no differences observed between doses or sexes. The majority of the radioactivity was recovered in the feces (82.4-92.7% of the administered dose); males appeared to excrete a greater proportion in the first 24 h (75.7-77.5%), while females excreted closer proportions during 0-24 h (44.1-49.9%) and 24-48 h (34.8% for both doses). Approximately 29.7-40.2% of the administered dose was extracted from the feces collected from 0-48 h in the low dose animals, while 48.1-57.2% was extracted from the high dose group. Urine accounted for a smaller percentage of the dose (4.7-9.0%), with females appearing to excrete a greater proportion (8.3-9.0%) compared to males (4.7-5.6%). The majority of radioactivity was accounted for in both excreta during the first 48 h. Cage wash accounted for 0.03-0.08% of the dose, while the combined tissues and carcass accounted for ≤0.36% of the administered dose.

The test compound was not extensively retained by any tissue. At 120 h post-dose, radioactivity was detected in the kidneys at both doses, with females retaining more radioactivity than males at both the low (0.144 vs 0.077 µg Eq/g) and high (4.42 vs 2.27 µg Eq/g) doses. Radioactivity was also detected in the thyroid of the high dose females (0.65 µg Eq/g), but was below the limit of detection in the high dose males and at the low dose. High dose females demonstrated greater overall retention and distribution of radioactivity. Whole blood and plasma demonstrated approximately equal amounts of radioactivity, indicating radioactivity was not partitioned into red blood cells. In all other tissues (excluding low residual levels in the stomach of the low and high dose males), the concentration of radioactivity was less than plasma levels or below the limit of accurate detection.

The test compound was rapidly absorbed, as radioactivity was detected in plasma at 0.25 h (first time point analyzed). At 1.5 mg/kg, a mean maximum plasma concentration (C_{max}) of 0.23 µg equivalents/mL was observed after two h (T_{max}) in the males; a C_{max} of 0.19 µg equivalents/mL was observed after four h in the females (Table 3). The $AUC_{96\text{-}120}$ was 3.3 µg equivalents•h/mL for the males and 2.9 µg equivalents•h/mL for the females. The half life ($t_{½}$) was longer in females (57.2 h) than in the males (44.1 h). At 50 mg/kg, C_{max} was much greater in the females (9.3 µg equivalents/mL) than in the males (2.8 µg equivalents/mL), both occurring at a T_{max} of 12 h. Similarly, the $AUC_{96\text{-}120}$ was 168.3 µg equivalents•h/mL in the females compared to 66.5 µg equivalents•h/mL in the males. The $t_{½}$ could not be calculated for the females at this dose.

Differences in the increase in administered dose compared to increases in C_{max} and AUC, along with the observed increase in T_{max} suggested that dose-dependent kinetics occurred for both the rate and extent of absorption of the test compound in both male and female rats.

Bile was a major excretion pathway. In bile duct-cannulated males, total recovery of the administered dose was 93.4-104%, with 8.7-18.4% of the administered dose recovered in the bile. Urine accounted for 4.4-5.5% of the administered dose, while feces accounted for 63.7-85.9% of the dose. The carcass accounted for 3.5-5.2%, and minor amounts of radioactivity (≤1.8%) were found in the GI tract and the cage wash. From these data, it can be calculated that approximately 23% of the administered dose was absorbed by the 1.5 mg/kg males, while only approximately 14% of the administered dose was absorbed by the 50 mg/kg males.

Animals dosed with donor bile directly into the duodenum demonstrated radioactivity in the urine and bile, indicating enterohepatic recirculation was taking place. In these males, total recovery of the administered dose was 108%, with 26.5% of the administered dose recovered in the bile. Urine accounted for 6.2% of the administered dose, while feces accounted for 74.6% of the dose. The carcass accounted for <15.1%, and minor amounts of radioactivity (<1.1%) were found in the GI tract and the cage wash.

HPLC analysis of urine, bile, and feces revealed broadly similar patterns of metabolites, with up to 18 fractions resolved. Parent was present in the fecal extracts at 4.3-7.5% of the administered dose in the 1.5 mg/kg group, and at 19.8-23.9% in the 50 mg/kg group. Parent was not present in the urine or bile. No fraction accounted for >5% of the administered dose in urine or bile, with the exception of fraction 6, which accounted for 5.0% of the administered dose in bile from the enterohepatic recirculation study. Fraction 6 was observed in urine, feces, and bile, and was confirmed as the monoglutathione conjugate of Chlorothalonil by TLC co-chromatography. Fractions 7, 12, and 13, generally present in urine, feces, and bile, were identified by LC/MS as mercapturic acid conjugates. Analysis following treatment of urine and bile with β-glucuronidase/sulphatase showed no evidence of deconjugation. It was stated that a component representing up to 4.8% of the dose in fecal extracts was not retained by HPLC and could not be further separated or characterized.

Pools of liver, kidney, and plasma were extracted and analyzed by HPLC. The extracts represented 50.0-59.3% of the liver radioactivity, 39.1-47.7% of the kidney radioactivity, and 72.7-78.1% of the plasma radioactivity. Fraction profiles were generally similar across all groups.

This metabolism study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OPP 85-1] in rats.

C. **STUDY DEFICIENCIES:** No deficiencies were noted.

ATTACHMENT

Pages 12-16 of the study report
(MRID 45710216)

Chlorothalonil DER (MRID# 45710216)

Page _____ is not included in this copy.

Pages __17__ through __21__ are not included in this copy.

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.

__X__ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

_____ Proprietary information pertaining to the chemical composition of an inert ingredient provided by the source of the ingredient.

_____ Attorney-Client Privilege.

_____ Claimed Confidential by submitter upon submission to the Agency.

_____ Internal Deliberative Information.

---

* 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.