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DATA EVALUATION RECORD

CHLOROTHALONIL

Metabolism in Rats

STUDY IDENTIFICATION: Savides, M.C., Marciniszyn, J.P., and Killeen, J.C., Jr. Pilot study of the effect of the gamma-glutamyl transpeptidase inhibitor, AT-125, on the metabolism of ^{14}C-chlorothalonil. (Unpublished study No. 1376-86-0072-AM-002 by Ricerca, Inc., Painesville, OH, for Fermenta ASC Corp., Mentor, OH; undated.) MRID No. 412505-06.

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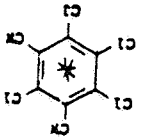
1. CHEMICAL: Terachloroisophthalonitrile; 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile; 1,3-dicyano-2,4,5,6-tetrachlorobenzene; chlorothalonil.

2. TEST MATERIAL: [^{14}C]Chlorothalonil, uniformly labeled in the benzene ring, and unlabeled chlorothalonil were used. The radiolabeled test material had a specific activity of 134.8 mCi/mmol and a radiochemical purity of 99 percent. Analytical-grade unlabeled chlorothalonil was 98.9 percent pure. The structure and radiolabel position (*) of [^{14}C]chlorothalonil are shown below:



3. STUDY/ACTION TYPE: Metabolism in rats.

4. STUDY IDENTIFICATION: Savides, M.C., Marciniszyn, J.P., and Killeen, J.C., Jr. Pilot study of the effect of the gamma-glutamyl transpeptidase inhibitor, AT-125, on the metabolism of ^{14}C-chlorothalonil. (Unpublished study No. 1376-86-0072-AM-002 by Ricerca, Inc., Painesville, OH, for Fermenta ASC Corp., Mentor, OH; undated.) MRID No. 412505-06.

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7. CONCLUSIONS:

A. Groups of three male rats were given an intraperitoneal injection of either AT-125, an inhibitor of gamma-glutamyl transpeptidase (GGT), or phosphate-buffered saline (PBS) 1 hour before administration of a single oral dose of 50 mg [^{14}C]chlorothalonil/kg. (The first step in the metabolism of glutathione conjugates involves GGT.)

Gastrointestinal absorption of [^{14}C]chlorothalonil was low and roughly equivalent for both groups of animals, accounting for about 7.4 percent of the administered dose at 48 hours postdosing. Levels of radioactivity in the blood, liver, and kidneys also were low, representing no more than 0.2, 0.21, and 1 percent of the ^{14}C dose, respectively, at 24 hours after administration of the test material. Nearly all (76 to 83 percent) of the ^{14}C in the blood was associated with the plasma. Approximately 80 percent and more than 50 percent of the radiolabel in the plasma and kidneys, respectively, were bound to proteins.

Pretreatment with AT-125 had no effect on the amount of radioactivity (as ppm, total ^{14}C, and percent of ^{14}C dose) in the blood, plasma, red blood cells, and liver; the GGT inhibitor also did not alter the rate or total amount of ^{14}C eliminated in the urine. However, at 24 hours postdosing, the kidneys of pretreated rats contained approximately three times more radioactivity than the kidneys of nonpretreated animals (48 ppm, 105 g ^{14}C equivalents, and 0.88 percent of the ^{14}C dose for AT-125-pretreated rats versus 17 ppm, 39 g, and 0.33 percent for nonpretreated rats); in addition, 70 percent of the radiolabel in the kidneys of rats pretreated with AT-125 was extracted into buffered saline (pH 7), compared with 44 percent from nonpretreated animals. Extractability of radiolabel in the urine collected for up to 12 hours also differed: only 15 percent was extracted from acidified urine, whereas more than 75 percent was extractable from the urine of rats given only chlorothalonil. Extractability of radiolabel from urine collected between 12 and 24 hours was similar (≥60 percent) for both groups.

The nonextractable fractions of all urine samples contained two metabolites, tentatively identified as the di- and triglutathione (GSH) conjugates of chlorothalonil, which are probable precursors of the urinary thiol metabolites of chlorothalonil. A third, unidentified (but less polar, based on elution time) metabolite was isolated from all nonpretreated rats and from the 12- to 24-hour urine samples of AT-125-dosed animals. AT-125-pretreated rats may have also excreted a metabolite containing four

glutathione moieties, and nonpretreated rats eliminated relatively large amounts of up to four additional (less polar) metabolites. For pretreated rats, the major metabolites were the diGSH and triGSH conjugates, which accounted for about 2 and 1.8 percent of the ^{14}C dose, respectively; between 11 and 45.5 percent of the urinary ^{14}C was the diGSH, and 18 to 41 percent of the urinary radiolabel was the triGSH conjugate. The major metabolite excreted in the urine of rats given only [^{14}C]chlorothalonil was not identified and represented approximately 2.71 percent of the ^{14}C dose and between 26 and 40 percent of the urinary radioactivity.

The data indicate that AT-125 affected the in vivo metabolism of [^{14}C]chlorothalonil by slowing the rate of conversion of initial metabolites to the polar products by increasing the amount of radioactivity in the kidneys at 24 hours after dosing. The study supports the prominent role of the glutathione pathway in the metabolism of chlorothalonil.

B. This study provides supplementary information on the metabolism of a single oral dose of 50 mg [^{14}C]chlorothalonil/kg in male rats. This was a pilot study and it does not meet EPA Guidelines (85-1) for the following reasons: (1) the use of only males; (2) the administration of only one dose level of chlorothalonil, rather than single low, single high, and repeated low doses; (3) no collection and analysis of feces; and (4) incomplete tissue distribution data.

Items 8 through 10--see footnote 1.

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[1]Only the items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1) Chlorothalonil labeled uniformly with ^{14}C in the benzene ring had a specific activity of 134.8 mCi/mmol and a radiochemical purity of 99 percent. Analytical-grade unlabeled chlorothalonil was 98.9 percent pure. Methods used to determine specific activity and purity were not specified. Lot and/or batch numbers also were not reported.

2) Male CD Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Portage, MI), weighing between 221 and 246 g at the time of dosing, were used. Animals were quarantined for at least 7 days before dosing; rats were housed individually in stainless steel cages during the acclimation period.

3) The chlorothalonil dosing suspension was prepared by dissolving [^{14}C]chlorothalonil and unlabeled chlorothalonil in methylene chloride, evaporating the solvent, and then adding a "small" amount of 0.75 percent methylcellulose to the remaining solid material. The mixture was shaken, the material pulverized (mean particle size 3.4 microns), and the suspension diluted with 0.75 percent methylcellulose to a final concentration of 5.19 mg/mL (44.95 Ci/mL; 2,30 mCi/mmol); the radiochemical purity of the [^{14}C]chlorothalonil in the dosing suspension was 97.1 percent.

The AT-125 dosing solution was prepared by dissolving 10.0 mg AT-125 in 2 mL 0.9 percent saline/0.005 M phosphate buffer (PBS).

4) After an overnight fast, rats (three/group) received an intraperitoneal (ip) injection of either 10 mg buffered AT-125/kg or PBS. At 1 hour after ip dosing, animals in both groups were given a single oral (intubation) dose of 50 mg [^{14}C]chlorothalonil/kg. One additional rat received no treatment and served as a control. Immediately following administration of the radiolabeled test material, control and experimental rats were placed in individual metabolism cages containing water but no food. Urine was collected over dry ice at 6, 12, and 24 hours after oral dosing. Cages were rinsed with water at each collection time. All rats were killed at 24 hours after administration of [^{14}C]chlorothaonil; the liver and kidneys were removed, blood was collected, and carcasses were stored frozen.

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5) Aliquots of blood were combusted and then analyzed for [^{14}C]CO_2 by liquid scintillation counting (LSC). The remaining blood was centrifuged to separate plasma from red blood cells. Aliquots of plasma were assayed directly by LSC; aliquots of red blood cells were oxidized before counting. Plasma was analyzed further by adding 10 percent trichloroacetic acid (TCA); supernatants were counted directly, and precipitated material was assayed for ^{14}C content following digestion/solubilization with pepsin (pH 2).

Liver and kidney samples were combusted and analyzed for radioactivity by LSC. In addition, kidney tissue from one animal of each group was homogenized and extracted three times with buffered saline (pH 7), saline (pH 2), methanol, ethyl acetate, and hexane.

The ^{14}C content of each extract was determined via LSC. Aliquots of the pH 7-buffered saline extract were treated with a fourfold volume of 10 percent TCA, and TCA-soluble material was counted. The TCA precipitate was digested twice with pepsin (pH 2), and pepsin-solubilized material was assayed for radioactivity by LSC. Solids remaining after pepsin digestion were solubilized in KOH and counted.

Aliquots of urine were analyzed directly for ^{14}C content by LSC. The remaining urine was frozen and stored for metabolite identification. Prior to analysis for chlorothalonil metabolites, individual urine samples were thawed, acidified to pH 2, and extracted with ethyl acetate. Individual samples were then analyzed for metabolites with reverse-phase high-performance liquid chromatography (HPLC) and LSC. After this analysis, individual samples were pooled according to treatment group, collection time, and extractability, and reanalyzed by reverse-phase HPLC and LSC.

B. Protocol: The protocol for this study is presented in the Appendix.

12. REPORTED RESULTS:

A. Rats pretreated with AT-125 received an average (± S.D.) ip dose of 9.96 ± 0.04 mg AT-125/kg; both AT-125-pretreated and PBS-pretreated rats received an average oral dose of 50.38 ± 0.69 mg [^{14}C]chlorothalonil/kg (101.80 ± 3.74 μCi). The method(s) used to make these determinations were not described.

B. One AT-125-pretreated rat had loose stools after administration of chlorothalonil, and all three rats dosed with this enzyme inhibitor had red coloration in the abdominal cavity at necropsy. No other remarkable clinical observations were reported.

C. The concentrations and percent recoveries of radioactivity in the blood, plasma, and red blood cells were similar for both AT-125-pretreated and vehicle-only pretreated rats (Table 1). The average (± S.D.) amount of radiolabel in the blood was 13.0 ± 3.6 μg, which represented approximately 1 percent of the ^{14}C dose; the mean ^{14}C concentration in blood was 871 ± 224 ppm. Red blood cells contained much smaller amounts (about 400 ppb ^{14}C) of radioactivity, and it was determined that plasma radioactivity accounted for about 80 percent of the radiolabel in the blood. Of the ^{14}C recovered from the plasma of both groups of rats, only 8 percent was soluble following protein precipitation with TCA; the remaining radioactivity (about 93 percent of the plasma ^{14}C) was recovered following pepsin digestion of TCA precipitates.

The amount of radioactivity in the liver, measured as total (absolute) amount of ^{14}C, concentration (ppm), and percent of ^{14}C dose, was slightly increased after pretreatment with AT-125, when compared with livers of vehicle-only treated rats (Table 2). Average values for these parameters were 17.6 μg, 1.53 ppm, and 0.150 percent, respectively, for all AT-125-pretreated animals. The amount of radioactivity recovered from the kidneys of AT-125-pretreated rats was approximately 2.7 times greater than that in the kidneys of rats that received only chlorothalonil (Table 2). Kidneys of pretreated rats contained a total of about 105 μg equivalents, which represented 0.88 percent of the ^{14}C dose; mean concentrations of radioactivity averaged 48 ppm. Kidneys of rats pretreated with only the vehicle contained less than 40 μg equivalents, or about 0.33 percent of the ^{14}C administered. The average ^{14}C residue concentration in the kidneys of these animals was 17 ppm. Approximately 70 percent of the radiolabel in the kidney tissue of one AT-125-pretreated rat was extracted into pH 7-buffered saline, compared with 44 percent from the kidneys of an animal administered only [^{14}C]chlorothalonil. Individual extracts with other solvents (saline, pH 2; methanol; ethyl acetate; hexane) contained no more than 2 percent of the radiolabel in either pretreated or nonpretreated kidney samples and accounted for a total of less than 4 percent when combined (CBI p. 47). Approximately 42 to 44 and 33.5 to 38 percent of the saline-soluble radioactivity in the kidneys of AT-125-pretreated and untreated rats, respectively, was soluble in 10 percent TCA; nearly all (81.5 to 97 percent) of the ^{14}C in the protein that precipitated following TCA

TABLE 1. Mean (± S.D.) Percent Recovery, Distribution, and Concentration of Radioactivity in the Blood of Rats 24 Hours After Oral Administration of [^{14}C]Chlorothalonil

Dosing Group[a]	^{14}C in blood: ppm[b]	Total µg equiv[c]	Percent of dose[d]	ppm ^{14}C (plasma)[e]	ppb ^{14}C (RBC)[f]	Percent in plasma[g]
AT-125-pretreated	899 ± 213[h]	13.4 ± 2.8	0.114 ± 0.028	1818 ± 552	352 ± 123	82.8 ± 8.4
Vehicle-pretreated	843 ± 318	12.5 ± 4.9	0.107 ± 0.041	1331 ± 303	403 ± 29	76.3 ± 4.0
All animals	871 ± 244	13.0 ± 3.6	0.111 ± 0.032	1574 ± 479	378 ± 85	79.6 ± 6.9

[a]AT-125-pretreated rats received an ip dose of 10 mg AT-125/kg 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg. Vehicle-pretreated rats received an ip injection of phosphate-buffered saline 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg.

[b]Ng ^{14}C equivalents/mL blood.

[c]Equiv, equivalents.

[d]Percent of the ^{14}C dose recovered from the blood.

[e]Ng ^{14}C equivalents/mL plasma.

[f]Ng ^{14}C equivalents/g red blood cells (RBC).

[g]Percent of radiolabel in the blood found in the plasma.

[h]Each value represents three male rats, except "all animals" values, which represent six rats.

Source: CBI Table 2, CBI p. 43.

TABLE 2. Mean (± S.D.) Percent Recovery and Concentration of Radioactivity in the Liver and Kidney of Rats 24 Hours After Oral Administration of [^{14}C]Chlorothalonil

Dosing Group[a]	Liver ^{14}C			Kidney ^{14}C		
	ppm[b]	Total (mg)[c]	Percent of ^{14}C dose	ppm[b]	Total (µg)[c]	Percent of ^{14}C dose
AT-125-pretreated	1.80 ± 0.48[d]	19.27 ± 4.95	0.164 ± 0.047	47.75 ± 4.81	104.75 ± 19.59	0.884 ± 0.140
Vehicle-pretreated	1.26 ± 0.39	15.95 ± 4.82	0.136 ± 0.038	17.20 ± 3.83	38.75 ± 10.29	0.331 ± 0.080
All animals	1.53 ± 0.49	17.61 ± 4.73	0.150 ± 0.041	--[e]	--	--

[a]AT-125-pretreated rats received an ip dose of 10 mg AT-125/kg 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg. Vehicle-pretreated rats received an ip injection of phosphate-buffered saline 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg.

[b]µg ^{14}C equivalents/g tissue.

[c]Total ^{14}C equivalents (mg, liver; µg, kidney).

[d]Each value represents three male rats, except for "all animals" values, which represent six animals.

[e]Not calculated.

Source: CBI Tables 4 and 5, CBI pp. 45 and 46.

treatment was solubilized by pepsin (CBI p. 48). Less than 5 percent of the saline (pH 7)-extracted radioactivity from the kidneys of AT-125-pretreated rats remained after pepsin digestion; approximately 7.5 to 10 percent was present after pepsin treatment of samples from nonpretreated rats.

D. Both pretreated and nonpretreated rats eliminated similar amounts of the ^{14}C dose at each collection interval (0 to 6 hours, 2 percent; 6 to 12 hours, 3 percent; 12 to 24 hours, 2.3 percent) and during the first 24 hours after administration of [^{14}C]chlorothalonil (7.4 percent) (Table 3). AT-125-pretreated rats eliminated slightly more radioactivity in the urine than nonpretreated rats during the first 6 hours after dosing (2.54 and 1.78 percent, respectively); the opposite was reported for the 6- to 12-hour postdosing collection period (2.54 and 3.39 percent, respectively).

Only 15 percent of the radiolabel in the acidified (pH 2) 0- to 6-hour and 6- to 12-hour urine samples of AT-125-pretreated rats was extracted into ethyl acetate; in contrast, more than 75 percent was extractable from the urine collected at 6 and 12 hours from rats given only [^{14}C]chlorothalonil (Table 4). Extractability of ^{14}C from urine collected between 12 and 24 hours after chlorothalonil administration was similar (60 versus 68 percent, respectively).

E. Three major peaks of radioactivity isolated (by HPLC/LSC) from individual urine samples accounted for 76.2 percent of the nonextractable urinary radiolabel and 66 percent of the total urinary ^{14}C excreted by AT-125-pretreated rats during the first 6 hours after administration of [^{14}C]chlorothalonil (CBI p. 51). Peaks eluting at 9 to 11, 5 to 7, and 2 to 4 minutes represented approximately 42, 15, and 9 percent of the urinary radioactivity, respectively (CBI p. 51). The 9- to 11-minute peak corresponded in elution time to a standard of the diglutathione (diGSH) conjugate of chlorothalonil; this peak also cochromatographed with the diGSH standard during a subsequent HPLC analysis. In addition, incubation with gamma-glutamyl transpeptidase (GGT) caused the amount of this metabolite (when isolated from a 6- to 12-hour urine sample) and the standard to decrease, and their elution times to change (per HPLC analysis). HPLC profiles of the radioactivity associated with the 5- to 7-minute peak--run before and after GGT treatment--also demonstrated a reduction in the amount of product in the presence of the enzyme. The 2- to 4-minute

TABLE 3. Mean Percent Recovery of Radioactivity in the Urine of Rats After Oral Administration of [^{14}C]Chlorothalonil

Dosing Group[a]	Percent of ^{14}C administered			
	0–6 hr[b]	6–12 hr	12–24 hr	0–24 hr
AT-125-pre-treated	2.54 ± 0.41	2.54 ± 0.70	2.41 ± 0.49	7.49 ± 0.14
Vehicle-pretreated	1.78 ± 1.52	3.39 ± 2.26	3.39 ± 2.26	7.31 ± 0.97
All animals	2.16 ± 1.08[d]	2.97 ± 1.56[d]	2.97 ± 1.56[d]	7.40 ± 0.63

[a]AT-125-pretreated rats received an ip dose of 10 mg AT-125/kg 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg. Vehicle-pretreated rats received an ip injection of phosphate-buffered saline 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg.

[b]Time after administration of [^{14}C]chlorothalonil.

[c]Each value represents three male rats, except for "all animals" values, which represent six rats.

[d]Values calculated by the reviewers.

Source: Adapted from CBI Table 8, CBI p. 49.

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TABLE 4. Extractability of Urinary Radioactivity into Ethyl Acetate Following Oral Administration of [^{14}C]Chlorothalonil to Rats

Dosing Group	Percent of total ^{14}C extracted in samples collected at: 0–6 hr[b]	6–12 hr	12–24 hr
AT-125-pretreated	14.8 ± 7.8[c]	15.5 ± 4.5	59.8 ± 16.3
Vehicle-pretreated	78.9	75.3 ± 1.8	68.3 ± 5.3

[a]AT-125-pretreated rats received an ip dose of 10 mg AT-125/kg 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg. Vehicle-pretreated rats received an ip injection of phosphate-buffered saline 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg.

[b]Time after administration of [^{14}C]chlorothalonil.

[c]Values represent the mean (± S.D.) of individual samples from three male rats, except for the 0- to 6-hour, vehicle-pretreated value, which represents only two rats.

Source: CBI Table 9, CBI p. 50.

peak was not affected by GGT treatment (chromatogram not presented). Results of the HPLC analysis of the extractable fractions of individual urine samples were not presented.

According to the study authors, differences in elution times of radiolabeled peaks from individual urine samples (both nonextractable and extractable fractions) were observed; they attributed these differences to replacement of chromatography columns during the course of HPLC analysis. Thus, remaining urine samples were pooled and reanalyzed for chlorothalonil metabolites by HPLC and LSC.

For both groups of rats, pooled nonextractable fractions of urine collected at 6 and 12 hours contained metabolites that eluted between 3 and 7 minutes and 8 and 12 minutes (Tables 5 and 6). The later-eluting (7- to 11-minute) peak from AT-125-dosed rats was tentatively identified as the diGSH conjugate of chlorothalonil. The earlier-eluting (3- to 7-minute) peak of animals pretreated with AT-125 may have consisted of two metabolites: one that eluted at 2 to 4 minutes in the HPLC analysis of individual samples, and a second that eluted at 5 to 7 minutes (tentatively identified as the triGSH conjugate of chlorothalonil). An additional peak with an elution time between 12 and 15 minutes was present in all nonextractable fractions of rats administered only chlorothalonil and in the nonextractable fractions of the 12- to 24-hour urine samples collected from AT-125-pretreated animals. This compound was not identified.

HPLC profiles of extractable urinary material from both groups of rats indicated major peaks of radioactivity at 12 to 15 and 18 to 21 minutes at each urine collection interval (Tables 5 and 6). Minor peaks in samples from AT-125-pretreated rats eluted between 2 and 4 minutes and 9 and 11 minutes; a minor peak isolated from the 0- to 6-hour urine of pretreated animals and the 6- to 12-hour urine of nonpretreated rats eluted at 22 to 24 minutes.

More than 75 percent of the nonextractable radiolabel from 0- to 6-hour and 6- to 12-hour urine samples of AT-125-pretreated rats was contained in two peaks: the 9- to 12-minute peak (the diglutathione conjugate of chlorothalonil), which represented approximately 43 (6 hours) and 28 (12 hours) percent of the total urinary radioactivity and 1.8 percent of the administered ^{14}C dose; and the 3- to 7-minute peak (possibly a mixture of the triGSH conjugate and another metabolite), which accounted for about 20 (6 hours) and 41 (12 hours) percent of the

TABLE 5. Reverse-Phase HPLC Quantification of Radioactivity in the Urine of Rats Injected Intraperitoneally with AT-125 Before Oral Dosing with [^{14}C]Chlorothalonil

Collection interval[a]	Elution time	Percent of NE fraction[b]	Percent of urinary ^{14}C	Percent of ^{14}C dose
0-6 hr	3-6 min	24.0[c]	20.2	0.53
	9-12 min	51.4	43.3	1.15
6-12 hr	3-7 min	50.5	41.0	0.88
	9-12 min	34.3	27.9	0.60
12-24 hr	3-5 min	40.9	17.9	0.43
	7-10 min	26.0	11.4	0.27
	13-15 min	15.7	6.9	0.17

Collection interval[a]	Elution time	Percent of EX fraction[d]	Percent of urinary ^{14}C	Percent of ^{14}C dose
0-6 hr	2-4 min	11.6	2.1	0.05
	9-11 min	12.1	2.2	0.06
	12-15 min	15.7	2.9	0.07
	18-21 min	20.3	3.7	0.09
	24-26 min	13.9	2.5	0.06
6-12 hr	2-4 min	5.1	0.8	0.02
	11-15 min	38.5	5.7	0.16
	18-21 min	28.0	4.2	0.11
12-24 hr	12-15 min	34.7	22.3	0.54
	18-21 min	41.8	26.9	0.65

[a]Time after administration of [^{14}C]chlorothalonil.

[b]NE, nonextractable.

[c]Values are for samples pooled from three animals.

[d]EX, extractable.

Source: CBI Table 11, CBI p. 52.

TABLE 6. Reverse-Phase HPLC Quantification of Radioactivity in the Urine of Rats Dosed with [^{14}C]Chlorothalonil

Collection interval[a]	Elution time	Percent of NE fraction[b]	Percent of urinary ^{14}C	Percent of ^{14}C dose
0-6 hr	3-6 min	24.0[c]	5.2	0.14
	8-11 min	43.3	9.3	0.25
	13-16 min	21.9	4.7	0.13
6-12 hr	3-7 min	25.8	6.6	0.22
	8-11 min	41.7	10.6	0.36
	12-15 min	21.8	5.6	0.19
12-24 hr	3-6 min	25.1	8.1	0.17
	8-11 min	32.5	10.5	0.23
	13-15 min	27.7	9.0	0.19

Collection interval[a]	Elution time	Percent of EX fraction[d]	Percent of urinary ^{14}C	Percent of ^{14}C dose
0-6 hr	11-6 min	42.7	35.0	0.94
	17-19 min	14.3	11.7	0.32
	19-21 min	28.7	23.5	0.63
6-12 hr	12-15 min	26.9	20.1	0.68
	17-21 min	43.6	32.6	1.10
	22-24 min	9.4	7.1	0.24
12-24 hr	12-15 min	39.9	27.3	0.58
	18-21 min	40.0	27.4	0.59

[a]Time after administration of [^{14}C]chlorothaonil.

[b]NE, nonextractable.

[c]Values are for samples pooled from three animals.

[d]EX, extractable.

Source: CBI Table 12, CBI p. 53.

urinary ^{14}C and 1.4 percent of the administered dose (see Table 5). The peaks eluting at 3 to 5 and 7 to 10 minutes from the 12- to 24-hour urine samples most likely corresponded, respectively, to the tri- and diglutathione conjugates described above. The additional peak (13- to 15-minute elution time) represented 6.9 percent of the urinary radioactivity and 0.17 percent of the ^{14}C dose and appeared to correspond to a peak in the nonextractable fractions of animals that did not receive AT-125 (see Table 6). When combined, the five metabolites isolated from the extractable fractions of urine collected from AT-125-pretreated rats through 6 hours postdosing accounted for approximately 13 percent of the urinary radioactivity and only 0.5 percent of the ^{14}C dose; the three metabolites in the 6- to 12-hour extractable fraction accounted for about 11 percent of the urinary radiolabel and 0.3 percent of the ^{14}C dose. In contrast, the 12- to 24-hour extractable urine fraction contained two peaks, each of which represented more than 20 percent of the urinary radioactivity and more than 0.5 percent of the ^{14}C administered.

For nonpretreated rats, three metabolites (elution times 3 to 7, 8 to 11, and 12 to 16 minutes) accounted for more than 85 percent of the nonextractable radiolabel in all urine collected (see Table 6). However, only the 8- to 11-minute peak represented more than 10 percent of the urinary radioactivity, and none accounted for more than 0.4 percent of the administered dose at any collection interval. These metabolites were considered minor and were not analyzed further. Approximately 80 percent of the extractable ^{14}C consisted of three peaks, each of which accounted for 7.1 to 35 percent of the urinary radioactivity and 0.24 to 1.10 percent of the ^{14}C dose for a given time interval. The study authors reported that these metabolites are being analyzed further and that results of these studies would be available in another report.

Evidence suggests that, in rats, AT-125 inhibits the metabolism of glutathione conjugates and, therefore, slows conversion to less polar metabolites (see Tables 7 and 8). During the first 12 hours after administration of chlorothalonil, AT-125-pretreated animals excreted much smaller amounts of less polar metabolites than nonpretreated rats; in contrast, the urine of both groups of rats contained roughly equivalent amounts of less polar products at 24 hours after compound administration. For pretreated rats, the major metabolites--each accounting for about 2 percent of the ^{14}C dose--eluted at 3 to 7 and 7 to 12 minutes; these compounds were tentatively identified as the tri- and diglutathione metabolites of chlorothalonil, respectively. For rats given only [^{14}C]chlorothalonil, peaks eluting between 11 and 16 and 17 and 21 minutes corresponded to approximately 2.71 and 2.64 percent of the

TABLE 7. Recovery and Distribution of Metabolites in the Urine of Rats Injected Intraperitoneally with AT-125 Before Oral Dosing with [^{14}C]Chlorothalonil[a]

Approximate Chromatographic Elution Time	Percent of ^{14}C in urine collected between: 0–6 hr[b]	6–12 hr	12–24 hr	Percentage of ^{14}C dose
2–4 min	2.1[c]	0.8	—[d]	0.07
3–7 min	20.2	41.0	17.9	1.84
7–12 min	45.5	27.9	11.4	2.08
11–15 min	2.9	5.7	29.2	0.94
18–21 min	3.7	4.2	26.9	0.85
24–26 min	2.5	—	—	0.06
Total	76.9	79.6	85.4	5.84

[a]Prepared by the study reviewers.

[b]Time after administration of [^{14}C]chlorothalonil.

[c]Values are for samples pooled from three animals. Metabolites from both extractable and nonextractable fractions were combined.

[d]No peak detected.

Source: Adapted from CBI Table 11, CBI p. 52.

TABLE 8. Recovery and Distribution of Metabolites in the Urine of Rats Dosed Orally with [^{14}C]Chlorothalonil[a]

Approximate Chromatographic Elution Time	Percent of ^{14}C in urine collected between: 0–6 hr[b]	6–12 hr	12–24 hr	Percent of ^{14}C dose
3–7 min	5.2[c]	6.6	8.1	0.53
8–11 min	9.3	10.6	10.5	0.84
11–16 min	39.7	25.7	36.3	2.71
17–19 min	11.7	—[d]	—	0.32
17–21 min	—	32.6	—	1.10
18–20 min	—	—	27.4	0.59
19–21 min	23.5	—	—	0.63
22–24 min	—	7.1	—	0.24
Total	89.4	82.6	82.3	6.96

[a]Prepared by the study reviewers.

[b]Time after administration of [^{14}C]chlorothalonil.

[c]Values are for samples pooled from three animals. Metabolites from both extractable and nonextractable fractions are included.

[d]No peak detected.

Source: Adapted from CBI Table 12, CBI p. 53.

^{14}C dose (the latter value may consist of up to four metabolites, none of which were identified). Other peaks of radioactivity accounted for <1 percent of the dose. All peaks combined represented between 78 and 95 percent of the ^{14}C dose recovered from the urine of pretreated and nonpretreated rats, respectively.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that the gamma-glutamyl transpeptidase (GGT) inhibitor, AT-125, affected the metabolism of chlorothalonil by altering the polarity of urinary metabolites for at least 12 hours and by increasing the amount of ^{14}C in the kidneys at 24 hours after oral dosing with [^{14}C]chlorothalonil.

AT-125 did not appear to affect concentrations of ^{14}C in the liver, blood, plasma, and red blood cells. For both AT-125-pretreated and nonpretreated rats, approximately 80 percent of the radioactivity in the blood was present in the plasma, and more than 80 percent of the plasma radioactivity was bound to proteins. Kidneys of AT-125-pretreated rats contained two to three times more radioactivity than kidneys of rats that received only chlorothalonil; approximately 70 percent of the radiolabel in the kidneys of rats pretreated with AT-125 was extracted into buffered (pH 7) saline, compared with 44 percent from nonpretreated animals. For both groups, more than 50 percent of the extracted radiolabel was bound to kidney proteins.

Pretreatment with AT-125 did not affect the rate of elimination of ^{14}C in the urine or the total amount of radioactivity excreted in the urine. However, the extractability of radiolabel in the urine varied considerably between the two groups. Only 15 percent of the radioactivity in the 0- to 6-hour and 6- to 12-hour urine samples (adjusted to pH 2) of pretreated rats was extractable with ethyl acetate, whereas more than 75 percent was extractable from acidified urine collected at 6 and 12 hours postdosing from rats given only chlorothalonil. Extractability of radiolabel from urine collected between 12 and 24 hours was similar (≥60 percent) for all groups of animals. The nonextractable fractions of all urine samples contained two metabolites, tentatively identified as diGSH and triGSH conjugates of chlorothalonil, probable precursors of the urinary thiol metabolites of chlorothalonil. A third unidentified (but less polar, based on a longer elution time) metabolite was isolated from all urine samples collected from nonpretreated rats and from the 12- to 24-hour urine samples of AT-125-dosed animals. Analysis of individual (rather than pooled) nonextractable radiolabel indicated

that AT-125-pretreated rats may have also excreted a metabolite containing four glutathione molecules or a metabolite not associated with glutathione conjugation.

The study authors concluded that AT-125 effectively inhibited the in vitro activity of GGT for a minimum of 12 hours. Evidence of this inhibition indicates the prominent role of the glutathione pathway in the metabolism of chlorothalonil and may suggest involvement of sulfur-containing metabolites in the nephrotoxicity of this fungicide.

B. A quality assurance statement and a statement of compliance with Good Laboratory Practices (GLPs), both signed and dated March 15, 1988, were included.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study provides supplementary information on the metabolism of orally administered [^{14}C]chlorothalonil and adequately describes the metabolism of a single oral dose of 50 mg [^{14}C]chlorothalonil/kg in male rats. This study also demonstrated that conjugation with glutathione is the major metabolic pathway for chlorothalonil in male rats. However, female rats were excluded (with no supporting rationale) from the study, and animals were given only one dose level of the test material, rather than a single low, single high, and repeated low doses as recommended by EPA (Guideline 85-1). Feces were not collected, and the only tissues analyzed for ^{14}C content were liver, blood, and kidneys; thus, absorption of [^{14}C]chlorothalonil was based on urinary elimination of radioactivity.

Gastrointestinal absorption of a single oral dose of [^{14}C]chlorothalonil was low, accounting for about 7.4 percent of the administered dose at 48 hours postdosing. Levels of radioactivity in blood, liver, and kidneys were low, representing no more than 0.2, 0.21, and 1 percent of the ^{14}C dose, respectively, at 24 hours after administration of the test material. As noted by the study authors, most radioactivity recovered from the plasma and kidneys was protein bound.

Pretreatment with AT-125 had no effect on the amount of radioactivity in the blood (including plasma and red blood cells) and liver; the GGT inhibitor also did not alter the rate or total amount of ^{14}C eliminated in the urine. However, AT-125 had a marked effect on kidney levels of radioactivity, which were about three times greater (as ppm, total residues, and percent of dose) in pretreated rats than in nonpretreated rats at 24 hours after [^{14}C]chlorothalonil administration. In addition, nearly twice as much of the radioactivity (i.e., 70

versus 44 percent) in the kidneys of pretreated rats was soluble in pH 7-buffered saline when compared with animals given only [^{14}C]chlorothalonil; extraction of radiolabeled renal material into organic solvents and pH 2-buffered saline was minimal from both groups. Pretreatment with AT-125 produced only a slight increase in the amount of radioactivity in the liver. The differences in ^{14}C concentrations between the kidney and liver--both with and without pretreatment with AT-125--are consistent with the relatively low levels of GGT in the bile duct cells of the liver, as contrasted with high GGT levels in kidney parenchymal cells.

Differences in the extractability of ^{14}C from the urine and in the metabolites excreted by pretreated versus nonpretreated rats were also observed. Most (63.5 to 69 percent) of the radioactivity in the 0- to 12-hour urine samples collected from pretreated rats was not extractable into organic solvent (ethyl acetate) at pH 2, whereas only 11 to 13 percent was extractable from these samples. A shift in extractability (i.e., to 36 percent nonextractable and 49 percent extractable) was observed for samples collected from pretreated rats between 12 and 24 hours postdosing, indicating the elimination of a greater amount of less polar metabolites during this time. In contrast, extractability of ^{14}C from the urine of nonpretreated rats was relatively high, accounting for approximately 55 to 70 percent of the urinary ^{14}C at all time intervals. The increase in the extractability of ^{14}C from the urine of pretreated rats at 12 to 24 hours after dosing was accompanied by an increase in the amount of metabolites with longer elution times (i.e., less polar compounds) and a general drop in shorter-eluting (i.e., more polar) metabolites.

The data indicate a retardation in the rate of conversion to less polar material by pretreated rats, an effect most likely resulting from AT-125-induced inhibition of GGT. The data support the conclusion of the study authors that AT-125, an inhibitor of GGT (the enzyme involved in the early steps of metabolism of glutathione conjugates) affected the metabolism of chlorothalonil by increasing the polarity of urinary metabolites for at least 12 hours after oral administration of [^{14}C]chlorothalonil; the increase in polarity was attributed, in part, to the blocking of glutathione metabolism or cleavage from chlorothalonil. As the inhibitory effect of AT-125 diminished, metabolism of the glutathione conjugates of chlorothalonil proceeded more readily, and these less polar metabolites, possibly thiols, as suggested by the study authors, were excreted in the urine. The study supports the integral role of glutathione in the metabolism of chlorothalonil.

Items 15 and 16 -- see footnote 1.

APPENDIX

Materials and Methods
(CBI pp. 63-73)

RIN 8587-93 EPA Reg II 50532-7

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