Metabolism of [*ring*-^{14}C]Ordram (Molinate) in the Rat. 1. Balance and Tissue Residue Study

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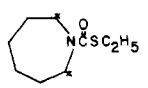
Excretion of ^{14}C after administration of [*ring*-^{14}C]Ordram, *S*-ethyl hexahydro[2-^{14}C]azepine-1-carbothioate, to rats was rapid, with approximately 97% of an oral dose (72 mg/kg) being excreted within 48 h after dosing. The major routes of excretion were via the urine (~88%, urine + cage wash) and feces (~11%). Less than 1% of the dose was detected in the sodium hydroxide air traps. No significant differences were observed between the rates and routes of excretion from male and female rats. Similarly, no significant differences in residual tissue ^{14}C attributable to sex effects were observed. With the exception of blood, residues associated with most tissues substantially decreased over the 7-day period after dosing. Whole-body residues decreased from an average of 13.8% of the administered ^{14}C at 1 day to 3.7% after 7 days. Seven-day blood residues were dose dependent, decreasing markedly from 40.1 ppm Ordram equivalents at a relatively high dose of 80 mg/kg to less than 1 ppm at a dose of 5 mg/kg.

Ordram (molinate) is a selective thiocarbamate herbicide used extensively in rice culture for the control of watergrass, *Echinochloa* spp., and other weed species (Herbicide Handbook, 1974). Rat metabolism studies were conducted to elucidate the biotransformation of [*ring*-^{14}C]Ordram in a mammalian species. This report deals with the balance and tissue residue phases of the study. A second report (DeBaun et al., 1978) describes the urinary metabolite characterization.

MATERIALS AND METHODS

Chemicals. [^{14}C]Ordram (*S*-ethyl hexahydro[2-^{14}C]-azepine-1-carbothioate) (8.2 mCi/mM) was provided by Stauffer Chemical Co., Richmond, Calif. (RRC). It was purified by preparative thin-layer chromatography (TLC) using 1 mm Analtech-GF chromatoplates developed in 2,2,4-trimethylpentane–*p*-dioxane (2:1). The final radiopurities as determined by TLC using 0.25-mm chromatoplates developed in 2,2,4-trimethylpentane–*p*-dioxane (2:1) and benzene–ether (7:3) were 98.7 and 97.9%, respectively. The dosing solutions were prepared by dissolving [^{14}C]Ordram and technical Ordram (99.1% AI) at a ratio of 1:26.9 in 1,2-propanediol (28.8 mg/mL).



[^{14}C]Ordram

* Denotes position of ^{14}C label

Treatment of Animals. *Balance Study.* Two female (184 and 194 g body weight) and two male (196 and 203 g body weight) Simonsen albino rats (Wistar derived) were fasted 24 h prior to administration of 0.5 mL of the [^{14}C]Ordram dosing solution by oral gavage. This dose (~72 mg/kg, ~50 × 10^6 dpm) is approximately one-tenth the oral LD_{50} value. After dosing, the rats were placed in glass metabolism cages, modified after Ford et al. (1966), which were designed for the separate collection of urine and feces. Air drawn through each cage was continuously flushed through a scrubbing tower containing 100 mL of 10% sodium hydroxide to trap any expired $^{14}CO_2$. Ground Purina Chow and water were available ad libitum for the duration of the study.

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Tissue Residue Study. Six female and six male Simonsen albino rats (~200 g) were dosed by oral gavage with [^{14}C]Ordram as described above. After dosing, the rats were placed in metabolism cages and provided with food and water ad libitum. Two female and two male rats were sacrificed by decapitation 1, 3, and 7 days after dosing, and organs and tissues were prepared for radioanalysis.

In order to determine the relationship between dose and residual ^{14}C in the blood, three male Simonsen albino rats (~200 g) were administered [^{14}C]Ordram orally at doses of 5, 20, or 80 mg/kg. The radiolabeled Ordram was diluted with technical material in 1,2-propanediol to provide 32.4 × 10^6 dpm in each 0.5-mL dose. Seven days after dosing, the animals were killed by cervical dislocation, and blood was removed by heart puncture for radioanalysis.

Sample Collection and Analysis. *Balance Study.* Urine samples were collected at 8, 24, 48, 56, and 72 h after dosing. Duplicate 10-μL aliquots were radioassayed directly by liquid scintillation counting (LSC) using 10 mL of Insta-Gel scintillation fluid (Packard Instrument Co.).

Feces were collected at 24, 48, and 72 h after dosing and were thoroughly mixed with a volume (mL) of water equal to one-half the weight (g) of the sample. Weighed aliquots (approximately 0.25 g) were combusted and radioassayed in triplicate using a Packard Model 305 Sample Oxidizer.

Sodium hydroxide air traps were sampled 4, 8, 24, 32, 48, and 72 h after dosing. Duplicate 0.5-mL aliquots were added to 3 mL of water and 15 mL of Insta-Gel for radioanalysis by LSC.

Cages were washed with approximately 300 mL of water after sample collection at the 24-, 48-, and 72-h intervals. The washes were homogenized with a Polytron homogenizer to disperse any suspended material and duplicate 1.0-mL aliquots in 10 mL of Insta-Gel were radioassayed by LSC.

All radioassays were performed using a Packard Model 3375 Tri-Carb scintillation counter with internal standardization.

Tissue Residue Study. Fat samples and total hide were digested in 10% sodium hydroxide for 2 weeks prior to homogenization in a Polytron homogenizer. An equal volume (mL/g) and 60 mL of the 10% sodium hydroxide were used for the fat and hide, respectively. The carcass, after removal of all other organs and tissues, was homogenized using a Universal hand-operated, meat and food chopper (ten successive passes). Triplicate aliquots of fresh

0021-8561/78/1426-1096$01.00/0

Table I. Distribution of ^{14}C in Excreta and Expired Air after Oral Administration of [^{14}C]Ordram (72 mg/kg) to Female and Male Rats

Sample analyzed	Interval, h	Percent administered dose: Female I	Female II	Male I	Male II	Average	Cumulative av.
Urine	8	22.2	46.7	32.8	13.0	28.7 ± 14.5	28.7 ± 14.5
	24	34.0	33.4	45.4	42.3	38.8 ± 6.0	67.5 ± 13.5
	32	3.5	1.3	2.2	9.0	4.0 ± 3.5	71.5 ± 11.1
	48	15.3	2.0	2.1	13.5	8.2 ± 7.2	79.7 ± 4.0
	56	3.1	0.1	0.2	0.9	1.1 ± 1 4	80.8 ± 2.7
	72	2.4	0.5	0.6	1.6	1.3 ± 0.9	82.1 ± 1.9
	Total	80.5	84.0	83.3	80.3		
Feces	24	8.8	8.8	5.9	10.2	8.4 ± 1.8	8.4 ± 1.8
	48	2.3	0.6	1.1	3.9	2.0 ± 1.5	10.4 ± 3.0
	72	0.3	0.1	0.1	0.3	0.2 ± 0.1	10.6 ± 3.1
	Total	11.4	9.5	7.1	14.4		
Expired air traps	4	0.1	0.2	0.2	0.2	0.2 ± 0.1	0.2 ± 0.1
	8	0.1	0.2	0.3	0.1	0.2 ± 0.1	0.4 ± 0.1
	24	0.2	0.4	0.4	0.2	0.3 ± 0.1	0.7 ± 0.2
	32	0.1	0.1	0.1	0.2	0.1 ± 0.1	0.8 ± 0.2
	48	0.1	0.0	0.0	0.1	0.1 ± 0.1	0.9 ± 0.2
	72	0.0	0.0	0.0	0.0	0.0 ± 0.0	0.9 ± 0.2
	Total	0.6	0.9	1.0	0.8		
Cage washes	24	9.2	4.4	1.6	5.5	5.2 ± 3.2	5.2 ± 3.2
	48	0.9	0.1	0.2	0.7	0.5 ± 0.4	5.7 ± 3.5
	72	0.1	0.1	0.1	0.1	0.1 ± 0.0	5.8 ± 3.5
	Total	10.2	4.6	1.9	6.3		
Accountability		102.7	99.0	93.3	101.8	*99.2* ± 4.2	

whole blood, fat homogenate, and hide homogenate were applied to cotton and frozen prior to combustion radioanalysis. Triplicate aliquots of carcass homogenate were treated similarly except that the samples were lyophilized prior to combustion.

All other organs and tissues were lyophilized and combusted directly. Weighed whole organs or organ sections not exceeding 0.5 g were placed on 4.25-cm filter paper in an aluminum weighing boat and were frozen prior to lyophilization. Contents of the stomach and intestines were removed by flushing with water prior to analysis. The combined gastrointestinal tract contents from each animal were homogenized in water (Polytron) and triplicate aliquots were analyzed for ^{14}C content after application to cotton, freezing, and lyophilization.

Radioanalysis was accomplished by combustion in a Packard Sample Oxidizer, Model 305, followed by liquid scintillation counting as described above. Combustion and counting efficiencies were computed daily prior to radioanalysis using [^{14}C]4-chlorobenzenesulfonic acid and [^{14}C]toluene as internal standards.

RESULTS AND DISCUSSION

Balance Study. Food consumption was normal and the animals remained healthy and active during the course of the study. As shown in Table I, the principal route of excretion was via the urine, a feature characteristic of most noncumulative pesticide chemicals (Matsumura, 1975). Approximately 80% of the dose was excreted by this route during the first 48 h after dosing. Another 10% of the dose was excreted in the feces during this interval. Less than 1% of the dose was accounted for in the expired air traps, indicating that minimal cleavage and degradation of the hexahydroazepine ring had occurred. Taking into account the ^{14}C present in the cage wash, approximately 97% of the dose was excreted within the 48-h interval.

Data in Table I also did not reveal a significant sex difference with regard to excretion rates and routes. The variability is actually greater within the female and male groups when compared to that observed between the sexes.

Table II. Tissue Distribution of ^{14}C in Rats Dosed Orally with [*ring*-^{14}C]Ordram (72 mg/kg)

Tissue	Ppm Ordram equivalents[a]: 1 day Female	1 day Male	3 day Female	3 day Male	7 day Female	7 day Male
Esophagus	11.8	7.0	3.0	3.5	2.7	2.6
Stomach	7.8	6.7	1.5	2.5	1.2	1.2
Small intestine (proximal)	28.1	24.8	0.6	1.9	3.3	0.9
Small intestine (distal)	11.1	7.5	1.9	2.4	1.3	1.6
Cecum	16.7	9.4	1.0	1.8	0.5	0.7
Liver	31.5	31.7	12.1	12.9	3.9	5.9
Kidney	21.0	22.0	10.3	9.0	3.3	4.4
Lungs	16.8	17.7	11.9	8.5	8.3	10.8
Heart	10.6	10.1	8.7	8.1	3.4	4.2
Spleen	15.4	15.6	9.3	9.5	5.6	6.4
Gonads	10.7	6.0	2.6	3.9	1.3	2.0
Muscle[b]	3.6	5.2	1.7	1.6	0.9	1.0
Bone[c]	3.8	4.5	1.4	1.7	0.7	1.0
Brain	5.0	5.3	3.4	3.8	2.2	1.3
Fat[d]	4.6	7.2	1.0	2.3	0.6	1.0
Hide	4.4	6.2	2.0	5.1	3.7	3.6
Carcass	5.4	6.9	2.8	2.8	1.4	1.5
Blood	39.9	30.9	34.5	30.8	26.9	29.7

[a] With the exception of 3-day female lung, 7-day female esophagus, and 7-day female spleen, for which data from only one animal is given, all other values are averages from two animals. [b] Gastrocnemius. [c] Femur. [d] Abdominal.

The average accountability is very good at 99.2%.

Tissue Residue Study. The distribution of ^{14}C in the tissues and organs of rats dosed orally with [^{14}C]Ordram is shown in Table II. No significant differences in residual tissue ^{14}C attributable to sex effects were evident from these data. Considering the rather large dose (72 mg/kg), initial tissue ^{14}C values are not inordinately high. In addition, the data demonstrate that the residues associated with most tissues and organs substantially decreased over the 7-day period after dosing. A notable exception is the rather high level in the blood which remains relatively

Table III. Parts per Million Equivalents of Ordram in the Blood of Rats 7 Days after Administration of Oral Doses of [*ring*-^{14}C]Ordram

Rat	Sp act. dose dpm/mg	Dose, mg/kg	Equiv in blood, ppm	Equiv, dose, ppm mg^{-1} kg^{-1}
1	3.24×10^7	5	0.9	0.18
2	8.10×10^6	20	8.9	0.45
3	2.03×10^6	80	40.1	0.50

constant over this period. The elevated blood level may also contribute to rather high 7-day values of other "blood-rich" tissues such as liver, kidney, lungs, heart, and spleen.

As shown in Table III, the amount of ^{14}C present in blood 7 days after treatment is dose related. The ppm Ordram equivalent value is roughly 4.5 times greater after the 80 mg/kg dose as compared with that after the 20 mg/kg dose. At a dose of 5 mg/kg, the 7-day value is less than 1 ppm. Precipitation of erythrocytes by centrifugation showed that virtually all of the blood ^{14}C was associated with the precipitated cellular fraction.

Since the blood was analyzed 7 days after administration of the herbicide, it is doubtful that the ^{14}C residues are due to the presence of intact parent compound. Studies by Casida et al. (1975) and Lay et al. (1975) demonstrated that, after sulfoxidation, certain thiocarbamates are capable of carbamoylating nucleophilic sites such as sulfhydryl groups in biological molecules. A similar mechanism may be responsible for the persistent blood residues observed in these studies after the administration of relatively high doses.

Expressed as percent administered ^{14}C, the combined gastrointestinal tract contents accounted for an average of 3.9, 0.1, and 0.03% at the 1-day, 3-day, and 7-day intervals, respectively. The whole-body residues, including the gastrointestinal tract contents, accounted for an average of 13.8% (1 day), 5.2% (3 day), and 3.7% (7 day) of the administered ^{14}C. Blood collected during the decapitation procedure accounted for roughly 25% of this 7-day value. Residual blood not removed from the tissues during decapitation may make a significant contribution to the 7-day, whole-body residue.

These data show that, even at a relatively high dose of 72 mg/kg, a single oral dose of [*ring*-^{14}C]Ordram is rapidly excreted, resulting in the elimination of approximately 97% of the administered ^{14}C in 48 h. A second report (DeBaun et al., 1978) shows that extensive metabolism of Ordram occurred in these animals. Unchanged Ordram accounted for only 0.1% of the urinary ^{14}C.

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Received for review February 7, 1978. Accepted 17, 1978.

Metabolism of [*ring*-^{14}C]Ordram (Molinate) in the Rat. 2. Urinary Metabolite Identification

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Ordram (*S*-ethyl hexahydroazepine-1-carbothioate) is readily degraded by the rat to more polar products which are excreted primarily in the urine. Unchanged Ordram accounts for only 0.1% of the urinary ^{14}C after an oral dose (72 mg/kg) of [*ring*-^{14}C]Ordram. The major metabolic pathway involves sulfoxidation and conjugation with glutathione, giving rise ultimately to a mercapturic acid derivative which accounts for 35.4% of the urinary ^{14}C. Ring hydroxylation to give the 3- and 4-hydroxy-Ordram derivatives (0.8% free, 26.1% as *O*-glucuronides) represents another major metabolic route. Hydroxylation in the 2 position of the ring and subsequent ring cleavage represent a minor pathway. Hexamethyleneimine (14.6%) and 3- and 4-hydroxyhexamethyleneimine (10.3%) are major metabolites presumably formed by hydrolysis of sulfoxidized Ordram and its hydroxy derivatives. Although there are small quantitative differences, the metabolism of [*ring*-^{14}C]Ordram in female and male rats is qualitatively the same.

Ordram (molinate) is a selective thiocarbamate rice herbicide used throughout the world for weed control in rice culture (Ashton and Crafts, 1973; Tweedy and Houseworth, 1976).

Recently, several definitive studies were published delineating the rapid degradation of thiocarbamate herbicides, including Ordram, in plants and mammals (Casida et al., 1975a,b). Lay and Casida (1976) have shown that in rats the thiocarbamates and corresponding sulfoxides form *S*-(*N*,*N*-dialkylcarbamoyl)-*N*-acetylcysteine derivatives. Hubbell and Casida (1977) have shown that rats treated ip with 1.0 mmol/kg of Ordram excreted 2% of the dose as *N*-acetyl-*S*(hexahydroazepine-1-carbonyl)-L-cysteine, (mercapturic acid conjugate) in the urine. Larger amounts of this conjugate were recovered from thiocarbamates and thiocarbamate sulfoxides containing *N*,*N*-dialkylcarbamoyl moieties.

This report describes rat metabolism studies with [*ring*-^{14}C]Ordram which were designed to elucidate the total biotransformation of this thiocarbamate herbicide in a mammalian species, based on identification of urinary

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0021-8561/78/1426-1098$01.00/0