of the applied radiocarbon, respectively. Conversely, captafol and atrazine significantly increased the production of $^{14}CO_2$ to 56.9 ± 0.4% and 57.3 ± 3.3%, respectively. Ammonium sulfate in [^{14}C]glucose-treated soils, however, had no effect. Soil amendments apparently affected certain soil microorganisms that were responsible for the breakdown of [^{14}C]glucose to $^{14}CO_2$. It is conceivable, therefore, that the effects of soil amendments on the fate of fonofos or parathion in soils also took place via an effect on soil microorganisms, although different groups might have been involved.

Results obtained with the insecticides under the different environmental conditions do show that soil amendments affect the persistence, metabolism, and translocation of both fonofos and parathion in soil and plants but the nature of these effects was different for each insecticide and no general conclusions can be drawn. It appears that the effects of some amendments occurred via a direct effect on soil microorganism populations that in turn are responsible for the degradation of the insecticides. In addition, organic fertilizers such as cow manure and sewage sludge are not of a specific, fixed chemical and microbiological composition. Their qualitative and quantitative makeup could be dependent on the source of the fertilizer and possibly on the food of the producing organism at a particular time. The major fact, however, is that the soil amendments did in one way or another change the fate and metabolism of the insecticides under the experimental conditions employed. It is for these reasons that the behavior and fate of pesticide chemicals in the agricultural environment should be studied by taking various ecological factors into consideration.

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Short-Term Fate of Mirex and 2,8-Dihydromirex in Rats

Janice E. Chambers,* Roger S. Case, Earl G. Alley, and James D. Yarbrough

Adult female and male rats were given a single oral dose of 38 μg of either [^{14}C]mirex or 2,8-dihydro-[^{14}C]mirex. Less than 0.6% of the mirex and 0.1% of the 2,8-dihydromirex doses were excreted in the urine during the 2-week period in either sex. During the first 2 days, fecal elimination of greater amounts of mirex in both sexes indicated that less mirex than 2,8-dihydromirex was absorbed. Fecal elimination between 3 and 14 days was about 3% and 1–2% of the mirex and 2,8-dihydromirex doses, respectively. Both compounds accumulated in the highest concentrations in fat. Concentrations of radioactivity in a number of tissues declined between 1 and 2 weeks. The patterns of distribution and excretion of mirex and 2,8-dihydromirex were very similar, indicating that the latter, a dechlorinated derivative of mirex, is no more readily eliminated than is the parent compound.

Mirex is a chemically stable compound composed of a polycyclic carbon skeleton with all available valences occupied by chlorine atoms. It is extremely refractory to biodegradation but is degraded slowly photochemically to hydrogen and/or oxygen-substituted derivatives (Ivie et al., 1974a). Some of these photodecomposition products are more polar and more water soluble than mirex and are potentially more susceptible to biotransformation and excretion (Ivie et al., 1974c).

Mirex is extremely lipophilic and resistant to metabolism; therefore, it partitions readily into animal fat and has a high potential for bioaccumulation. The disposition and excretion of mirex have been studied in the laboratory rat, Japanese quail, mosquitofish, rhesus monkey, goat, and cow (Gibson et al., 1972; Mehendale et al., 1972; Dorough and Ivie, 1974; Ivie et al., 1974b,c; Pittman et al., 1976; Smrek et al., 1978). In these studies, mirex was retained for long periods in body fat, and it was excreted at low rates in the feces and in only trace amounts in the urine.

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Table I. Percent Elimination in Urine and Feces of Rats of a Single Oral Dose of 38 Micrograms of [^{14}C]Mirex or 2,8-Dihydro[^{14}C]mirex[a]

	mirex				2,8-dihydromirex			
	urine		feces		urine		feces	
sex	2 days	14 days	2 days	14 days	2 days	14 days	2 days	14 days
male	0.36	0.44	28.8	31.4	0.02	0.07	15.9	18.2
female	0.50	0.58	21.2	24.5	0.02	0.04	17.5	18.9

[a] Two-day values are the average of 10-12 rats; 14-day values are the average of 5-6 rats.

Metabolites were not detected in any species except the monkey in which a mirex metabolite was found in feces, but it was believed to be the result of the action of gut bacteria (Stein et al., 1976). A primary photoproduct of mirex, 8-hydromirex, has also been found to be lipophilic, resistant to metabolism, poorly eliminated in the feces, and eliminated in only trace amounts in the urine (Gibson et al., 1972; Hallett et al., 1978; Chu et al., 1979). Rats excreted polar photoproducts of mirex more rapidly than they excreted mirex or nonpolar mirex photoproducts (Ivie et al., 1974c). 2,8-Dihydromirex was accumulated similarly to mirex following iv administration and in limited studies following oral administration; there was no evidence for metabolism (Chu et al., 1980). An oxygenated analogue, chlordecone (Kepone), is similar to mirex and 8-hydromirex with regard to lipophilicity, elimination, and lack of metabolism, although this compound demonstrated a lesser tendency to accumulate in fat than did mirex (Egle et al., 1978).

The present studies were designed to determine whether dechlorination of mirex would make the compound any more susceptible to biological elimination. The tissue distribution and excretion of a dihydrogen derivative of mirex, 2,8-dihydromirex, and mirex itself were investigated in parallel studies during a 2-week period following a single oral dose in adult male and female rats. Although mirex and 2,8-dihydromirex are both relatively nonpolar, the two carbon–hydrogen bonds in the latter give it a greater potential for hydrogen bonding and consequently a greater potential for water solubility and excretion than mirex.

EXPERIMENTAL PROCEDURES

Uniformly labeled [^{14}C]mirex, specific activity 6.5 mCi/mmol, was obtained from California Bionuclear Corp. 2,8-Dihydro[^{14}C]mirex was synthesized from this [^{14}C]-mirex by a modification of the method of Kecher et al. (1974). Both compounds were dissolved in soybean oil to give an activity of 2.5×10^6 dpm/mL. The chemical purities of the [^{14}C]mirex and 2,8-dihydro[^{14}C]mirex used were 99% and 98%, respectively.

Adult Sprague-Dawley derived rats of both sexes, weighing between 150 and 250 g, were used. Each animal was housed individually in a plastic metabolism cage. Animals were acclimated to the metabolism cage for at least 1 day prior to dosing. Animals had access to ground laboratory feed (Wayne Lab Blox) and water ad libitum. Animals were dosed orally with 38 μg (10^6 dpm) of the test compound in 0.4 mL of oil, followed by 0.1 mL of oil. The average dosage was 0.17 μg/kg of body weight.

Urine and feces were collected daily for up to 2 weeks. Animals were sacrificed at the end of 1 or 2 weeks, and the following tissues were removed: brain stem, cerebellum, cerebrum, spinal cord, fat (pelvic, mesenteric, and subcutaneous), esophagus, stomach, duodenum, ileum, cecum, rectum, liver, lung, heart, kidneys, gonads, uterine horns, vagina plus cervix, epididymis plus vas deferens, adrenals, skeletal muscle, spleen, thymus, and diaphragm. Fecal samples were homogenized in methanol in a Sorvall grinder, and aliquots of the resultant slurry were used for quantitation of radioactivity. Urine, fecal, and tissue samples were combusted in a Packard Tri-carb sample oxidizer, Model P306, and radioactivity in the samples was quantified in a Packard Model 3255 liquid scintillation spectrometer. All samples except urine samples were counted to accumulate 2000 counts, which yielded a standard error of counting of less than 5%. There were five or six animals in each of the treatment groups. Data were calculated on a nanograms of mirex equivalent basis.

RESULTS

Patterns of elimination of both [^{14}C]mirex and 2,8-dihydro[^{14}C]mirex were similar. Only trace amounts of either compound appeared in the urine (Table I). By the end of 2 weeks, less than 0.6% of the mirex dose and less than 0.1% of the 2,8-dihydromirex dose had been excreted in the urine. In both sexes with both compounds, urinary excretion was much greater in the first week than in the second week (Table II).

The majority of radioactive material eliminated in the feces occurred within the first 2 days following dosing in both sexes with both compounds, followed by a gradual elimination during the remainder of the experimental period (Table I). Greater fecal elimination of [^{14}C]mirex than of 2,8-dihydro[^{14}C]mirex occurred in both sexes. Greater overall elimination of [^{14}C]mirex occurred in males than in females, 31% and 25%, respectively, at the end of 2 weeks, with the greatest difference occurring on the first day. After initial elimination, the rate of fecal excretion of [^{14}C]mirex was the same in both sexes, with excretion in the second week 2–3 times slower than excretion in the first week (Table II). Overall fecal elimination of 2,8-dihydro[^{14}C]mirex was similar between both sexes with 18–19% of the dose eliminated at the end of 2 weeks (Table I). After initial elimination, the rate of fecal excretion was somewhat higher in males and was constant during the remainder of the experimental period (Table II). This rate of excretion was of the same magnitude as [^{14}C]mirex excretion in the second week.

Accumulation of both compounds in nervous tissue was moderate to low and was generally higher in females than

Table II. Rates of Elimination of [^{14}C]Mirex or 2,8-Dihydro[^{14}C]mirex in Male and Female Rats following a Single Oral Dose of 38 Micrograms[a]

	mirex						2,8-dihydromirex					
	urine			feces			urine			feces		
sex	days	ng equiv/day	r^2	days	μg equiv/day	r^2	days	ng equiv/day	r^2	days	μg equiv/day	r^2
male	1-5	10.30	0.99	2-7	0.14	0.97	1-6	2.30	0.99	2-14	0.07	0.98
	6-12	0.32	0.85	8-14	0.04	0.93	6-14	1.20	0.95			
female	1-6	7.57	0.88	2-7	0.13	0.96	1-7	1.28	0.98	2-14	0.04	0.94
	7-14	0.32	0.89	8-14	0.05	0.98	7-14	0.20	0.82			

[a] Linear regressions were calculated on the averages of 10-12 rats for 1-7 days and 5-6 rats for 8-14 days.

Table III. Concentrations (Nanogram Equivalents per Gram of Tissue) of Radioactivity in the Nervous and Reproductive Systems of Rats Receiving a Single Oral Dose of 38 Micrograms of [^{14}C]Mirex or 2,8-Dihydro[^{14}C]mirex[a]

	female		male	
	7 days	14 days	7 days	14 days
	Mirex			
brain stem	58.5 ± 32.8 (6)	15.2 ± 5.4 (4)	21.7 ± 3.7 (5)	ND
cerebellum	44.1 ± 24.1 (6)	6.5 ± 1.8 (5)	13.2 ± 2.6 (5)	3.7 ± 2.3 (6)
cerebrum	40.4 ± 24.7 (6)	9.4 ± 2.5 (5)	13.4 ± 2.8 (5)	1.7 ± 1.1 (6)
spinal cord	48.4 ± 30.7 (6)	7.1 ± 3.7 (5)	28.0 ± 5.5 (5)	ND
gonad	253.3 ± 46.1 (4)	2.5 ± 1.3 (5)	11.6 ± 2.3 (5)	5.8 ± 1.2 (6)
uterine horns	15.4 ± 1.0 (4)	0.4 ± 0.4 (5)		
vagina and cervix	40.7 ± 14.5 (6)	13.6 ± 4.8 (5)		
epididymis and vas deferens			54.7 ± 5.6 (5)	59.3 ± 3.5 (6)
	2,8-Dihydromirex			
brain stem	25.0 ± 7.5 (6)	10.6 ± 1.3 (6)	14.7 ± 1.8 (5)	11.5 ± 3.2 (6)
cerebellum	22.9 ± 4.2 (6)	8.1 ± 1.5 (6)	11.8 ± 3.4 (4)	6.6 ± 2.3 (6)
cerebrum	16.4 ± 4.3 (6)	6.3 ± 1.1 (6)	11.1 ± 2.4 (5)	5.5 ± 1.7 (6)
spinal cord		24.9 ± 10.0 (6)	24.5 ± 5.3 (5)	14.7 ± 3.6 (6)
gonad	287.8 ± 66.1 (6)	76.1 ± 13.7 (6)	7.6 ± 3.7 (5)	3.2 ± 1.4 (6)
uterine horns	81.0 ± 18.0 (6)	21.6 ± 6.5 (6)		
vagina and cervix	47.6 ± 17.0 (6)			
epididymis and vas deferens			122.6 ± 36.0 (5)	125.3 ± 48.6 (6)

[a] Each value is the mean ± SE (*N*).

Table IV. Concentrations (Nanogram Equivalents per Gram of Tissue) of Radioactivity in Fat and Portions of the Gastrointestinal Tract of Rats Receiving a Single Oral Dose of 38 Micrograms of [^{14}C]Mirex or 2,8-Dihydro[^{14}C]mirex[a]

	female		male	
	7 days	14 days	7 days	14 days
	Mirex			
mesenteric fat	337.5 ± 63.2 (6)	1042.9 ± 368.0 (5)	160.5 ± 32.0 (5)	418.9 ± 89.1 (6)
pelvic fat	943.7 ± 405.3 (6)	540.0 ± 215.4 (5)	478.9 ± 92.6 (5)	486.2 ± 58.6 (6)
subcutaneous fat	594.6 ± 255.6 (6)	483.1 ± 159.1 (5)	420.6 ± 63.2 (5)	530.1 ± 57.7 (6)
esophagus	25.7 ± 5.7 (5)	7.4 ± 7.4 (4)	17.4 ± 6.6 (5)	3.0 ± 2.6 (6)
stomach	19.2 ± 3.3 (6)	24.0 ± 5.6 (5)	18.7 ± 4.1 (5)	5.5 ± 3.5 (6)
duodenum	16.2 ± 6.1 (6)	11.2 ± 2.3 (5)	7.2 ± 1.9 (5)	4.6 ± 2.1 (6)
ileum	20.6 ± 7.5 (6)	11.9 ± 2.4 (5)	14.0 ± 4.3 (5)	11.9 ± 4.5 (6)
cecum	16.6 ± 8.2 (6)	7.2 ± 2.0 (5)	6.9 ± 1.7 (5)	0.7 ± 0.5 (6)
rectum	11.7 ± 5.6 (6)	3.9 ± 1.2 (5)	4.1 ± 1.4 (5)	0.1 ± 0.1 (6)
	2,8-Dihydromirex			
mesenteric fat	556.2 ± 133.8 (6)	496.1 ± 79.2 (4)	573.7 ± 107.9 (5)	525.7 ± 123.9 (5)
pelvic fat	1115.5 ± 244.3 (6)	650.2 ± 141.4 (6)	740.8 ± 164.9 (5)	660.2 ± 175.3 (6)
subcutaneous fat	806.3 ± 163.9 (5)	566.9 ± 85.1 (6)	677.5 ± 149.2 (5)	621.6 ± 131.3 (6)
esophagus	83.0 ± 23.1 (6)	21.9 ± 5.8 (6)	58.2 ± 12.9 (5)	37.9 ± 12.8 (5)
stomach	81.4 ± 24.1 (6)	18.2 ± 0.5 (6)	21.0 ± 5.2 (5)	26.6 ± 4.9 (6)
duodenum	34.5 ± 9.0 (6)	10.6 ± 3.2 (6)	12.1 ± 4.5 (5)	8.5 ± 4.0 (6)
ileum	146.7 ± 38.9 (6)	50.2 ± 19.7 (6)	67.9 ± 30.1 (5)	10.3 ± 2.2 (6)
cecum	53.2 ± 19.7 (6)	26.9 ± 12.3 (5)	10.1 ± 2.6 (5)	13.8 ± 7.6 (6)
rectum	17.9 ± 4.0 (6)	49.0 ± 14.2 (6)	18.7 ± 7.3 (5)	10.6 ± 3.9 (6)

[a] Each value is the mean ± SE (*N*).

in males (Table III). Of all experimental groups, mirex accumulation in nervous tissue was highest in females at 7 days. Levels of radioactivity in all groups decreased from 7 to 14 days. Accumulation of both compounds was of a similar magnitude.

Accumulation of radioactivity from both compounds was high in ovaries at 7 days following dosing and probably reflects accumulation in the closely associated fat; levels were decreased at 14 days (Table III). Levels of both compounds were moderate to low in the female reproductive tract and they decreased with time; concentrations of 2,8-dihydromirex were greater than those of mirex. Concentrations of both compounds were low and comparable in the testes. As in female reproductive tissue, there was a decline in the levels of both compounds in the testes. Concentrations of both compounds were moderate in the male reproductive tract and remained constant with time. The concentrations in the male tract of 2,8-dihydromirex were higher than those of mirex. No distinction could be made among radioactivities associated with the tract itself, fat, or reproductive secretions.

Accumulation of both compounds was high in the fat samples and was generally higher in females than in males at 7 days after dosing, but concentrations were comparable between the sexes at 14 days after dosing (Table IV). There was generally a decline in concentrations of both compounds in females between 1 and 2 weeks; this trend was observed only with 2,8-dihydromirex in males. Concentrations of 2,8-dihydromirex were greater than those of mirex in both sexes.

Accumulation of both compounds in the gastrointestinal tract was generally low (Table IV). No consistent differences between the two sexes were apparent. A general decline in concentrations of radioactivity occurred between 7 and 14 days. Concentrations of 2,8-dihydromirex were typically higher than concentrations of mirex for comparable tissues in both sexes.

Concentrations of both compounds declined with time in all other tissues examined in females except 2,8-dihydromirex in skeletal muscle (Table V). Tissue concentrations of both compounds in males generally declined with time, but the trend was not as consistent as with

Table V. Concentrations (Nanogram Equivalents per Gram of Tissue) of Radioactivity in Selected Organs and Tissues of Rats Receiving a Single Oral Dose of 38 Micrograms of [^{14}C]Mirex or 2,8-Dihydro[^{14}C]mirex[a]

	female		male	
	7 days	14 days	7 days	14 days
		Mirex		
liver	53.5 ± 15.4 (5)	45.5 ± 3.4 (5)	102.7 ± 36.6 (5)	36.4 ± 4.4 (6)
lung	74.7 ± 11.4 (6)	46.5 ± 8.9 (5)	23.6 ± 4.2 (5)	52.9 ± 6.0 (6)
heart	58.7 ± 17.3 (6)	30.1 ± 8.5 (5)	16.7 ± 4.6 (5)	12.1 ± 1.6 (6)
kidney	61.3 ± 14.9 (6)	33.8 ± 5.3 (5)	17.3 ± 5.0 (5)	6.8 ± 2.3 (6)
diaphragm	66.5 ± 23.9 (6)	26.8 ± 7.1 (4)	73.5 ± 8.5 (5)	35.6 ± 7.8 (6)
adrenal	201.3 ± 64.3 (5)	137.1 ± 38.2 (5)	168.9 ± 39.2 (5)	69.6 ± 9.5 (6)
skeletal muscle	40.1 ± 12.7 (6)	20.2 ± 6.9 (5)	8.1 ± 1.8 (5)	15.4 ± 3.6 (6)
spleen	26.9 ± 3.9 (6)	17.8 ± 2.1 (5)	6.7 ± 1.7 (5)	14.1 ± 4.5 (6)
thymus	61.3 ± 24.8 (5)	60.5 ± 15.0 (4)	17.4 ± 2.7 (5)	48.1 ± 16.6 (6)
		2,8-Dihydromirex		
liver	39.9 ± 6.7 (6)	18.5 ± 2.2 (6)	51.1 ± 11.1 (4)	51.9 ± 18.3 (6)
lung	108.2 ± 33.8 (6)	82.1 ± 9.5 (6)	72.4 ± 14.7 (5)	31.9 ± 4.6 (6)
heart	14.7 ± 3.7 (6)	8.6 ± 0.7 (6)	9.9 ± 3.7 (5)	8.0 ± 2.0 (6)
kidney	24.5 ± 4.9 (6)	10.0 ± 2.9 (6)	17.9 ± 4.7 (5)	9.1 ± 2.8 (6)
diaphragm		32.0 ± 11.8 (6)	56.3 ± 28.8 (5)	20.5 ± 5.1 (6)
adrenal	360.8 ± 81.2 (6)	94.2 ± 10.8 (6)	136.6 ± 30.5 (5)	65.5 ± 18.1 (6)
skeletal muscle	1.9 ± 0.8 (4)	16.6 ± 4.1 (6)	11.5 ± 3.5 (5)	16.7 ± 5.0 (6)
spleen	27.8 ± 9.3 (6)	4.0 ± 1.5 (6)	9.9 ± 5.7 (4)	6.5 ± 2.9 (6)
thymus	25.1 ± 7.4 (5)	15.2 ± 3.8 (6)	52.2 ± 10.8 (5)	10.2 ± 2.6 (6)

[a] Each value is the mean ± SE (N).

Table VI. Average Percent of Radioactivity of Initial Dose Retained in Selected Tissues of Male and Female Rats 2 Weeks after Dosing with 38 Micrograms of [^{14}C]Mirex or 2,8-Dihydro[^{14}C]mirex[a]

	mirex		2,8-dihydro-mirex	
organ	female	male	female	male
brain	0.03	<0.01	0.04	0.04
digestive tract	0.16	0.07	1.06	0.41
liver	1.35	1.42	0.51	1.41
lung	0.24	0.26	0.38	0.23
heart	0.06	0.04	0.02	0.02
kidney	0.16	0.04	0.06	0.05
gonad	<0.01	0.04	0.02	0.02
reproductive tract	<0.01	0.12	0.02	0.26
adrenal	0.02	<0.01	0.02	<0.01
diaphragm	0.05	0.06	0.05	0.03
spleen	0.02	0.03	<0.01	<0.01
thymus	0.09	0.10	0.02	0.02
total	2.18	2.18	2.20	2.49

[a] Each value is the average of five to six rats.

females; concentrations of both compounds in skeletal muscle increased with time in males. There were no consistent trends in distribution between sexes or between compounds in these tissues. Although the concentrations of both compounds are relatively high in some small tissues, such as adrenal glands, the percentage of total dose retained in small (Table VI).

DISCUSSION

Similar excretion patterns were observed for both mirex and 2,8-dihydromirex. Neither compound was excreted to an appreciable extent in the urine. The majority of fecal elimination of both compounds occurred during the first 2 days following dosing and represented material that was not absorbed; this elimination was lower for 2,8-dihydromirex than that for mirex, indicating that the former was absorbed in greater quantities. Although females absorbed mirex in greater amounts than males did, this was probably not related to the greater fat content of females into which the compound partition, because essentially the same amount of 2,8-dihydromirex was absorbed by both sexes. There appear to be differences in rates of urinary and fecal excretion after 2 days between the sexes and between compounds; however, these rates of elimination are very small and would have little effect on body loads.

Although lipid solubility of both compounds is similar, the presence of two hydrogen atoms in 2,8-dihydromirex should give it greater capacity for hydrogen bonding and, therefore, potentially greater water solubility. This property would assist it in being absorbed by being more readily solvated in the aqueous gut contents and, therefore, more easily transported to the absorptive surface of the intestinal epithelium. Our data are consistent with this interpretation.

The decline in radioactivity in most tissues between 1 and 2 weeks is in part the result of excretion of both compounds but could also represent a mobilization and redistribution into other tissues. Both compounds in males and 2,8-dihydromirex in females increased in concentration in skeletal muscle with time. Even though concentrations of radioactivity in skeletal muscle are low, the tissue accounts for a large proportion of the body mass and, therefore, could represent a potential reservoir for storage of these xenobiotics; however, the low concentrations of both compounds indicate that muscle is not storing large amounts of the doses. In fact, most of the tissues studied were not found to store a very significant proportion of the dose (Table VI). As expected, liver represented the discrete organ containing the highest percentage of the dose. Somewhat surprising was the lung, which contained the next highest percentage. As expected, fat samples accumulated the greatest concentrations of radioactivity, and therefore, adipose tissue represents the largest reservoir for both compounds.

The tissue distribution and excretion patterns were not dramatically different between the two compounds. Other researchers have confirmed that mirex is not metabolized (Gibson et al., 1972; Mehendale et al., 1972; Dorough and Ivie, 1974; Ivie et al., 1974b,c). The similarity in the behavior of 2,8-dihydromirex to that of mirex gave no suggestion that it had been metabolized and is consistent with the lack of metabolism reported by Chu et al. (1980).

Therefore, it appears that the 2,8-dihydrogen derivative of mirex is no more easily eliminated than mirex and, because of its slightly greater absorption, would actually be retained for longer periods of time than would the parent compound.

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Metabolism of a Herbicide, Isouron [3-(5-*tert*-Butyl-3-isoxazolyl)-1,1-dimethylurea], in Bean Seedlings

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The matabolism of isouron [3-(5-*tert*-butyl-3-isoxazolyl)-1,1-dimethylurea] was studied in bean plants. Two major degradation pathways, N-demethylation and hydroxylation of the *tert*-butyl group, seemed to be followed. The following four metabolites were identified by cochromatography and mass spectrometry: a monomethylurea derivative [3-(5-*tert*-butyl-3-isoxazolyl)-1-methylurea], a urea derivative [3-(5-*tert*-butyl-3-isoxazolyl)urea], a hydroxy-*tert*-butyl dimethylurea derivative [3-[5-(1,1-dimethyl-2-hydroxyethyl)-3-isoxazolyl]-1,1-dimethylurea], and a hydroxy-*tert*-butyl monomethylurea derivative [3-[5-(1,1-dimethyl-2-hydroxyethyl)-3-isoxazolyl]-1-methylurea]. In addition, three polar metabolites were tentatively identified, which were β-D-glucosides of 3-(5-*tert*-butyl-3-isoxazolyl)-1-(hydroxymethyl)-1-methylurea and hydroxy-*tert*-butyl dimethylurea and hydroxy-*tert*-butyl monomethylurea derivatives.

Isouron is a newly synthesized isoxazole–urea derivative that shows a potent herbicidal activity against many species of annual broadleaf and grassy weeds and some perennial weeds (Yukinaga et al., 1979a). It has been used for control of the total vegetation in noncropland (Ito et al., 1979) and has shown promising selective herbicidal activity in sugarcane field (Yukinaga et al., 1979b). Isouron was reported to inhibit the Hill reaction in isolated spinach chloroplasts; thus the primary site of its action was suggested to be in the photosynthetic electron transport system (Yukinaga et al., 1979c).

Differences among plant species in metabolic rates and pathways of herbicides often contribute to the selectivity of their weed control, and identification of their metabolites is very important in analyzing residues left in crops. The present investigation was conducted to determine the metabolic fate of isouron in bean plants.

MATERIALS AND METHODS

Chemicals. Isouron and its related compounds were synthesized at Shionogi Research Laboratories, Fukushima-ku, Osaka, Japan. [^{14}C]Isouron labeled at the 5 position of the isoxazole ring was also prepared at the laboratories and had a specific activity of 5.03 mCi/mmol. Its radiochemical purity as determined by thin-layer chromatography (TLC) was greater than 99.3%.

Plant Material. Seeds of the kidney bean (*Phaseolus vulgaris* L. cv. "Black Valentine") were surface sterilized with a 0.1% (w/v) sodium hypochlorite solution for 15 min and soaked in running tap water for 3 days at 25 °C. The germinated seeds were planted in moist sand and grown under greenhouse conditions with a 28 °C daytime and 15 °C nighttime temperature, without artificial light. A few days before the primary leaves fully expanded, the cotyledons were removed from the plants. Seedlings were then transferred to water culture in a Hoagland's nutrient solution (Hoagland and Arnon, 1938) and allowed to grow for 3 days prior to root treatment. The nutrient solution was aerated for 30 min at 6-h intervals during growth.

Application of [^{14}C]Isouron. All experiments were carried out under greenhouse conditions. Lots of five uniform seedlings were selected, and each lot was supplied with [^{14}C]isouron through the roots by being placed in 30 mL of a 10^{-4} M solution in a test tube. After 4 h the plants were removed from the test tube, and their roots were rinsed 3 times with 20 mL of distilled water. The plants then were cultured in 100 mL of a fresh nutrient solution

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