JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Metabolism of a New Herbicide, [^{14}C]Pyribenzoxim, in Rice

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ABSTRACT: The *in vivo* metabolism of a new herbicide pyribenzoxim (benzophenone O-[2,6-bis(4,6-dimethoxypyrimidin-2-yloxy)benzoyl]oxime) in rice was carried out using container trials. Two radiolabeled forms of [carbonyl-^{14}C]pyribenzoxim (**P1**) and [ring-^{14}C(U)]pyribenzoxim (**P2**) were treated separately as formulations for foliar treatment by single applications of 50 g of active ingredient (ai)/ha at the 4−6 leaves stage. At 0, 7, 30, and 60 days after treatment (DAT), samples of panicle, foliage/rest of plant, and roots were taken for analysis. Upon harvest (120 DAT), rice plants were separated into grain, husk, straw, and root parts. Total radioactive residues (TRRs) at each sampling date were determined to show that the final radioactive residues at harvest were low in grain, husk, straw, and roots, accounting for <17 ppb. The concentration of final residues in the rice plant decreased rapidly, and less than 0.1% of initial TRRs remained at harvest. At 7 DAT, metabolite 1 [**M1**, 2,6-bis(4,6-dimethoxypyrimidin-2-yloxy)benzoic acid] and two unknown compounds (other-1 and other-2) were detected in foliage extract, accounting for 3.5% TRRs (21.0 ppb), 3.1% TRRs (19.0 ppb), and 9.0% TRRs (54.3 ppb), respectively, while 26.1% of **M1** was observed in solvent wash. Any other metabolites were not detected in the plant, including expected metabolite **M3** (benzophenone oxime). On the basis of the results obtained, a metabolic pathway of pyribenzoxim in a rice plant was proposed.

KEYWORDS: Pyribenzoxim, herbicide, plant metabolism, TRRs, rice

INTRODUCTION

Pyribenzoxim (benzophenone O-[2,6-bis(4,6-dimethoxypyrimidin-2-yloxy)benzoyl]oxime),[1,2] a new pyrimidynyloxybenzoic herbicide analogous to pyrithiobac,[3−6] bispyribac-sodium,[7−9] pyriminobac-methyl,[10,11] and pyriftalid,[12] was developed by LG Chemical, Ltd., Korea, for post-emergence treatment in rice fields. Similar to sulfonylurea herbicides, this compound was known as an inhibitor of acetolactate synthase (ALS), involved in the biosynthesis of the branched-chain amino acids in plants,[13,14] and showed a maximal level of inhibition in whole plants within 24 h after treatment.[15] No phytotoxicity was observed, and low acute toxicity (rats, oral) of >5000 mg/kg was reported.[1,16] The LC_{50} for common carp (*Cyprinus carpio* L.) in 96 h was >10 mg/L. The log *P* measured by the shake flask method is 3.04, while the solubility (25 °C) in water is 3.5 mg/L and the vapor pressure (25 °C) is $<7.4 \times 10^{-5}$ mmHg.[16,17] The bioconcentration factor (BCF) was 33.2 for common carp (*C. carpio*), suggesting that the possibility of biocentration was very low in the aquatic environment.[18] In sandy loam soil, pyribenzoxim degraded rapidly, with the half-life of 1.3 days, suggesting that the possibility of leaching and accumulation in lower soil and groundwater is very low, and the major metabolites formed were 2,6-bis(4,6-dimethoxypyrimidin-2-yloxy)benzoic acid and 2-hydroxy-6-(4,6-dimethoxypyrimidin-2-yloxy)benzoic acid.[19] However, only limited information has been available on the environmental fate and metabolism of pyribenzoxim.

Plants are the major ultimate recipients of pesticides, from either direct application, soil uptake, or atmospheric drift. Pesticide may remain on the surface of plants or may penetrate the cuticle of leaves, fruits, stem, and roots by virtue of their lipophilicity.[20] Many pesticides have been shown to be taken up into the plant mainly through the leaf surface, fruits, and roots.[21−23] Plant metabolism studies of pesticides are very important for predicting the degradation behavior of the parent pesticide and determining the nature and extent of the metabolites as well as assessing the potential human, animal, and environmental hazards.

In the present investigation, a container trial experiment was conducted to measure the levels of total radioactive residues, quantify the proportion of the residues, assess residue distribution, identify major metabolites, and obtain a biotransformation pathway in rice.

MATERIALS AND METHODS

Chemicals. The radiolabeled test compounds [carbonyl-^{14}C]-pyribenzoxim (**P1**) and [ring-^{14}C(U)]pyribenzoxim (**P2**), non-radiolabeled

Received: October 10, 2010
Accepted: January 11, 2011
Revised: January 5, 2011
Published: February 10, 2011

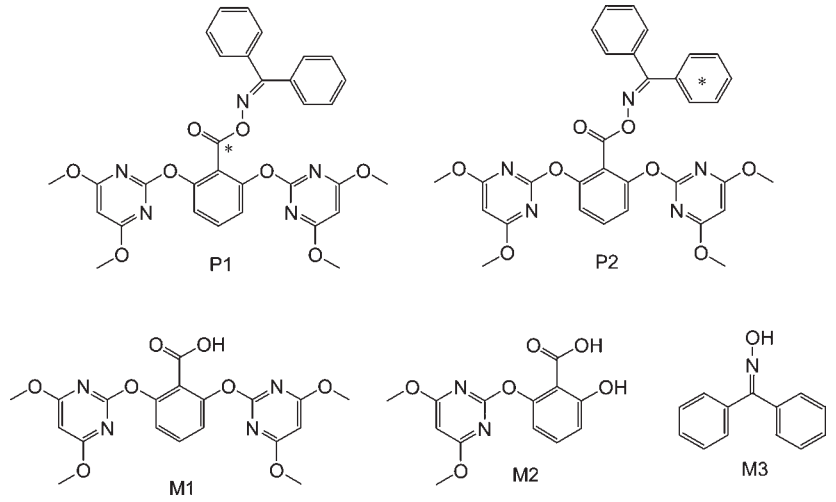

Figure 1. Structures of [carbonyl-^{14}C]pyribenzoxim (**P1**), [ring-^{14}C(U)]pyribenzoxim (**P2**), and metabolites (**M1**, **M2**, and **M3**). The site of the ^{14}C label is marked with an asterisk.

Table 1. Physicochemical Properties of the Test Soil

pH[a]	organic carbon (%)[b]	cation-exchange capacity (meq/100 g)	sand (%)[c]	silt (%)[c]	clay (%)[c]	texture (USDA)
5.4	2.4	9.2	41.2	46.8	12.0	silt loam

[a] Measured in 1:5 soil-deionized water suspension.[20] [b] Walkley−Black colorimetric determination.[21] [c] Particle-size analysis = hydrometer method.[22]

Table 2. Summary of Crop Sampling Schedule and Fresh Weight of Each Part

		fresh weight (g) (mean[a] ± SD[b])	
sampling time	matrix	^{14}C-carbonyl (**P1**)	^{14}C-ring (**P2**)
application	foliage	1.1 ± 0.3	NS[c]
	roots	0.6 ± 0.1	NS
7 DAT[d]	foliage	6.8 ± 1.0	7.4 ± 1.5
	roots	10.9 ± 0.6	12.7 ± 1.0
intermediate (30 DAT)	foliage	96.9 ± 2.5	NS
	roots	95.6 ± 5.3	NS
intermediate (60 DAT)	panicle	15.0 ± 2.5	15.1 ± 3.6
	rest of plant	149.1 ± 28.3	119.5 ± 16.0
	roots	107.4 ± 25.3	81.7 ± 6.1
	straw	109.7 ± 10.3	98.1 ± 9.2
harvest	roots	77.8 ± 14.2	68.4 ± 13.3
	grain	23.6 ± 2.1	22.1 ± 0.7
	husk	5.8 ± 0.8	5.0 ± 0.3

[a] Mean = mean of triplicate. [b] SD = standard deviation of triplicate determination. [c] NS = no sample at this time point. [d] DAT = days after treatment.

Table 3. TRRs in Rice Plant

		TRRs (ppb, mean[a] ± SD[b])	
sampling time	matrix	^{14}C-carbonyl (**P1**)	^{14}C-ring (**P2**)
application	foliage	23791.3 ± 10009.5	NS[c]
	roots	85.2 ± 18.8	NS
7 DAT[d]	foliage	603.6 ± 253.2	1329.9 ± 397.4
	roots	8.9 ± 1.0	13.4 ± 6.0
intermediate (30 DAT)	foliage	15.8 ± 5.1	NS
	roots	1.9 ± 0.3	NS
intermediate (60 DAT)	panicle	11.8 ± 3.8	12.8 ± 5.0
	rest of plant	1.6 ± 0.9	0.9 ± 0.0
	roots	2.5 ± 0.8	2.1 ± 0.4
	straw	1.6 ± 0.1	1.3 ± 0.2
harvest	roots	3.1 ± 0.8	2.4 ± 0.4
	grain	2.9 ± 0.5	2.3 ± 0.1
	husk	8.8 ± 1.6	9.9 ± 0.6

[a] Mean = mean of triplicate. [b] SD = standard deviation of triplicate determination. [c] NS = no sample at this time point. [d] DAT = days after treatment.

pyribenzoxim, and its metabolites, 2,6-bis(4,6-dimethoxypyrimidin-2-yloxy)benzoic acid (**M1**), 2-hydroxy-6-(4,6-dimethoxypyrimidin-2-yloxy) benzoic acid (**M2**), and benzophenone oxime (**M3**), were kindly provided by LG Chem Investment, Korea (Figure 1). The radiochemical purities were >98%, and specific activities of **P1** and **P2** were 3.37 and 3.40 MBq/mg, respectively. Both compounds were used without further purification. High-performance liquid chromatography (HPLC)-grade acetone, dichloromethane, acetonitrile, water, and methanol were purchased from Burdick and Jackson (Muskegon, MI). All of the other reagents and common chemicals were of analytical grade.

Test System. The rice variety was Chucheong, a typical Japonica short-grain type, and the soil (silt loam) characteristics are shown in Table 1.[24−26] Portions of rice seed were soaked in running water for approximately 3 days (pigeon breast stage) and sown on the nursery boxes, which were filled with soil (passed through a 2 mm sieve) to a depth of 2 cm. The nursery boxes were placed in a greenhouse maintained at 25−28 °C and watered regularly for 2 weeks. A plastic pot ($^1/_{3000}$ *a* dimension) was filled to a depth of 25 cm with soil and flooded to a depth of 3 cm before treating with fertilizer. A few days after, three clumps of rice seedlings from the nursery boxes were transplanted into the flooded soil in the middle of the pots at the 4−6 leaves stage. The rice pots were maintained in a flooded condition for the test period. The rice pots were inspected at regular intervals to monitor water levels and plant growth and determine the treatment of any fertilizer and crop

dx.doi.org/10.1021/jf1039469 |J. Agric. Food Chem. 2011, 59, 1918–1923

protection agents for healthy growth. These plants were carefully watered to avoid wash-off, and some of the applied radioactivity remained on the treated leaves throughout the experiment period, potentially allowing for continued cuticular uptake of these residues.

Preparation and Foliar Treatment of [^{14}C]Pyribenzoxim Formulation. A nominal 7.5 mg of ^{14}C-pyribenzoxim was added to 0.15 mL of blank emulsifiable concentrate (EC) formulation (supplied by the sponsor) to make 5% EC. Then, the formulation was diluted by 500-fold with distilled water to make 75 mL of spray solution. The nursery boxes for each treatment were placed in a hood and enclosed in a aluminum frame covered with polyethylene to confine the sprayed formulation. The formulated [^{14}C]pyribenzoxim (about 0.57 MBq) was sprayed over the plants using a hand-held aerosol sprayer at a field use rate of 0.05 kg/ha.[27] After 2 h, the rice plants from nursery boxes were transplanted into the plastic pots.

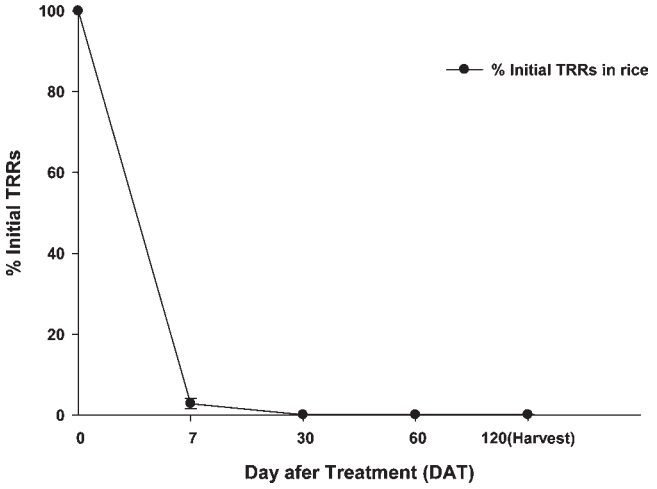

Figure 2. TRRs of [carbonyl-^{14}C]pyribenzoxim in a rice plant.

Sampling and Preparation. At each sampling time, rice plants of triplicate pots were sampled. Rice plants were cut off just above the soil surface and separated into commodities appropriate to the growth stage at sampling. Roots were removed from the soil and washed with water to remove soil as much as possible, and the washings were discarded. The fresh weights of samples were measured (Table 2). Each compartment was surface-washed by rinsing with 50−300 mL of acetonitrile. The total volume of washed solution was measured, and triplicate aliquots (1 mL) were taken for liquid scintillation counting (LSC). If necessary, the washed solution was concentrated prior to LSC analysis. The plant samples were stored frozen (< −15 °C) prior to homogenization, in which samples were cut into small pieces and blended in the presence of dry ice until they were reduced to fine particles. The dry ice was allowed to evaporate at room temperature, and the sample was reweighed. To determine concentrations of radioactivity, triplicate subsamples (0.2−0.5 g) were taken for combustion and analysis of LSC.

Radioassay. Radioactivity of all liquid samples was measured by LSC, using model LS 6000TA (Beckman, Brea, CA) liquid scintillation counter with external quench correction. Hionic fluor (5 mL) was used for the aqueous samples, and Insta-fluor (10 mL) was used for organic samples. The non-extractable bound residue (200 mg) was combusted by an oxidizer (Packard model 307, Boston, MA) after mixing with Combustaid (100−200 μL). The [^{14}C]carbon dioxide produced was absorbed in Carbo-sorb E (5 mL) and mixed with Permafluor E^{++} scintillation cocktail (10 mL) for LSC.

Chromatography. Determination of radioactivity and identification of pyribenzoxim and metabolites were performed on the Thermo Separation Product model P2000 HPLC system equipped with an ultraviolet−visible (UV−vis) detector and a radioactivity monitor (Packard Radiomatic 150TR, 500 μL/min liquid cell) by co-chromatography with

Table 4. Distribution of [Carbonyl-^{14}C]pyribenzoxim (P1) and Its Metabolites in the Surface Wash of Foliage/Rest of Plant and in Foliage/Rest of Plant/Straw at the Each Sampling Time

	application		7 DAT[a]		30 DAT		60 DAT		harvest	
component	ppb	% TRR[b]	ppb	% TRR	ppb	% TRR	ppb	% TRR	ppb	% TRR
surface wash										
pyribenzoxime	23201.6 ± 9631.6	97.52	246.3 ± 106.4	40.8	3.8 ± 1.4	24.1	ND[c]	NA[d]	NS[e]	NA
M1	ND	NA	157.3 ± 50.1	26.1	ND	NA	ND	NA	NS	NA
M2	ND	NA	ND	NA	ND	NA	ND	NA	NS	NA
other-1	ND	NA	ND	NA	ND	NA	ND	NA	NS	NA
other-2	ND	NA	ND	NA	ND	NA	ND	NA	NS	NA
surface wash total	23,201.6 ± 9631.6	97.52	403.6 ± 155.3	66.9	3.8 ± 1.4	24.1	0.3 ± 0.1[f]	18.8	0.02 ± 0.00[g]	1.3
extracts										
solvent extractable										
pyribenzoxime	585.8 ± 507.3	2.46	13.2 ± 22.8	2.2	2.7 ± 3.7	17.1	0.7 ± 0.7	43.7	ND	NA
M1	ND	NA	21.0 ± 8.5	3.5	ND	NA	ND	NA	ND	NA
M2	ND	NA	ND	NA	ND	NA	ND	NA	ND	NA
other-1	ND	NA	19.0 ± 15.4	3.1	ND	NA	ND	NA	ND	NA
other-2	ND	NA	54.3 ± 14.1	9.0	ND	NA	ND	NA	ND	NA
subtotal	585.8 ± 507.3	2.46	107.5 ± 57.8	17.8	2.7 ± 3.7	17.1	0.7 ± 0.7	43.7	0.50 ± 0.05[d]	32.1
water layer	0.0 ± 0.0	0.0	40.3 ± 19.2	6.7	5.6 ± 0.7	35.4	0.0 ± 0.0	0.0	0.00 ± 0.00	0.0
extractable total	585.8 ± 507.3	2.46	147.8 ± 77.0	24.5	8.3 ± 3.5	52.5	0.7 ± 0.7	43.7	0.50 ± 0.05	32.1
bound residue	3.9 ± 2.8	0.02	52.2 ± 23.3	8.6	3.7 ± 1.6	23.4	0.6 ± 0.3	37.5	1.04 ± 0.10	66.6
TRRs	23,791.3 ± 10,009.5	100.0	603.6 ± 253.2	100.0	15.8 ± 5.1	100.0	1.6 ± 0.9	100.0	1.56 ± 0.10	100.0

[a] DAT = days after treatment. [b] TRRs = total radioactivity residues. [c] ND = not detected. [d] NA = not applicable. [e] NS = no sample for identification by radio-HPLC. [f] Estimated value by [carbonyl-^{14}C]pyribenzoxim-specific activity corresponding to $^{1}/_{2}$ instrumental detection limit (IDL). [g] Calculated value by LSC analysis.

dx.doi.org/10.1021/jf1039469 |J. Agric. Food Chem. 2011, 59, 1918–1923

Table 5. Radioactive Proportion of [Ring-^{14}C(U)]pyribenzoxim (P2) in the Surface Wash of Foliage/Rest of Plant/Straw and in Foliage/Rest of Plant/Straw at the Each Sampling Time

	7 DAT[a]		60 DAT		harvest	
component	ppb	% TRR[b]	ppb	% TRR	ppb	% TRR
surface wash						
pyribenzoxime	1203.9 ± 356.8	90.5	ND[c]	NA[d]	NS[e]	NA
M3	ND	NA	ND	NA	NS	NA
other-1	ND	NA	ND	NA	NS	NA
other-2	ND	NA	ND	NA	NS	NA
surface wash total	1203.9 ± 356.8	90.5	0.4 ± 0.1[f]	44.4	0.03 ± 0.02[g]	2.3
extracts						
solvent extracts						
pyribenzoxime	56.2 ± 17.7	4.2	ND	NA	ND	NA
M3	ND	NA	ND	NA	ND	NA
other-1	ND	NA	ND	NA	ND	NA
other-2	ND	NA	ND	NA	ND	NA
subtotal	56.2 ± 17.7	4.2	0.4 ± 0.0	44.4	0.56 ± 0.08	42.4
water layer	34.1 ± 28.0	2.6	0.0 ± 0.0	0.0	0.00 ± 0.00	0.0
extractable total	90.3 ± 45.7	6.8	0.4 ± 0.0	44.4	0.56 ± 0.08	42.4
bound residue	35.7 ± 6.4	2.7	0.1 ± 0.1	11.2	0.73 ± 0.11	55.3
TRRs	1329.9 ± 397.4	100.0	0.9 ± 0.0	100.0	1.32 ± 0.20	100.0

[a] DAT = days after treatment. [b] TRRs = total radioactivity residues. [c] ND = not detected. [d] NA = not applicable. [e] NS = no sample for identification by radio-HPLC. [f] Estimated value by [carbonyl-^{14}C]pyribenzoxim-specific activity corresponding to $^1/_2$ IDL. [g] Calculated value by LSC analysis.

authentic compounds. A reverse-phase Luna C_{18} column (4.6 × 250 mm, 5 μm, Phenomenex, Torrance, CA) was used. For the analysis of parent and metabolites, different mobile-phase conditions [90% acetonitrile in water containing 0.1% trifluoroacetic acid for the analysis of parent and 60% acetonitrile in water containing 0.1% trifluoroacetic acid for the analysis of metabolites] were employed for 10 min at the flow rate of 1.0 mL/min. UV detection (247 nm) was performed with a variable wavelength detector. Radioactivity monitoring was performed using scintillation cocktail (Ultima Flo-M, 2 mL/min). Under these conditions, the retention times of pyribenzoxim, **M1**, **M2**, and **M3** were approximately 5.0, 6.9, 5.1, and 7.8 min, respectively.

Extraction Efficiency. Triplicate samples (10 g) of homogenized foliage were weighed into Teflon centrifuge tubes (50 mL). **P1** (14.07 kBq), **M1**, **M2**, and **M3** in methanol (10 mg/L solution) was treated at a concentration of 1.0 mg/kg, and acetonitrile/water (1:1, v/v, 20 mL) was added. The sample was extracted by shaking at 300 rpm for 1 h and centrifuged at 3000 rpm for 20 min to separate the supernatant and residue. The supernatant was filtered through a glass fiber filter paper (pore size of 0.7 μm) to remove the precipitate. The residue was further extracted in the same way using acetonitrile (×2) and methanol (×1). The total extracts were reduced to less than 50 mL under vacuum at 40 °C and transferred to a separatory funnel. To the extract, 50 mL of saturated NaCl solution was added and pH was adjusted to approximately 2 using 1 mL of 10 N H_2SO_4 before partitioning with 100 mL of dichloromethane 3 times. The combined dichloromethane fractions were evaporated under vacuum at 40 °C to less than 10 mL and then concentrated to dryness under N_2 gas. The samples were then redissolved in 1 mL of methanol for HPLC analysis.

Analysis of Extractable and Non-extractable Samples. At sampling dates, triplicate samples of homogenized foliage, root, rest of plant, straw, grain, and husk were taken, and then extraction and analysis were performed by the method mentioned above in extraction efficiency. After the extraction and partition of the samples, the residual solid sample was dried and homogenized intensively. Triplicate samples were combusted for LSC analysis.

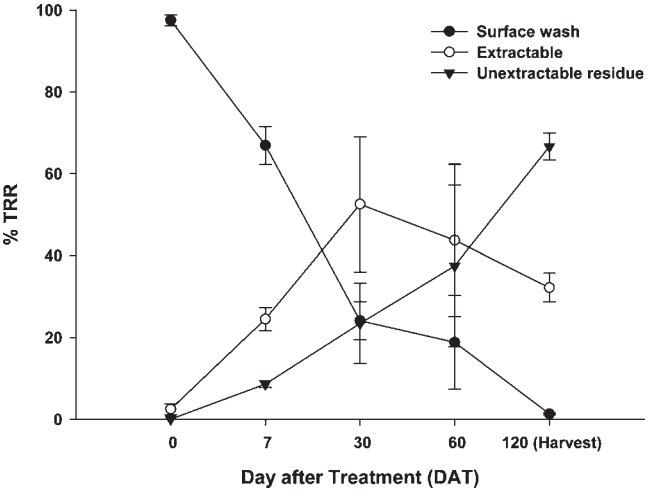

Figure 3. Percentage TRRs of [carbonyl-^{14}C]pyribenzoxim in the various solvent/extraction/unextractable fractions of rice plant.

RESULTS AND DISCUSSION

Extraction Efficiency. **P1**, **M1**, **M2**, and **M3** were recovered from rice plant with a high yield and reproducibility of 97.3 ± 6.2, 95.0 ± 3.6, 95.7 ± 3.7, and 95.2 ± 3.4%, respectively, indicating that good extraction method is established.

Total Radioactive Residues (TRRs). Radioactive residues in rice plants are summarized in Table 3. After foliar treatment of [carbonyl-^{14}C]pyribenzoxim (**P1**), TRRs in plants decreased rapidly from a total of 23 876 parts per billion (ppb) at 0 DAT to 17 ppb at harvest (DAT), remaining less than 0.1% of applied TRRs (Figure 2). Foliage accounted for the most of TRRs, showing 99.6% TRRs at application and 98.5% TRRs at 7 DAT. In roots, TRRs also dissipated quickly to give a similar

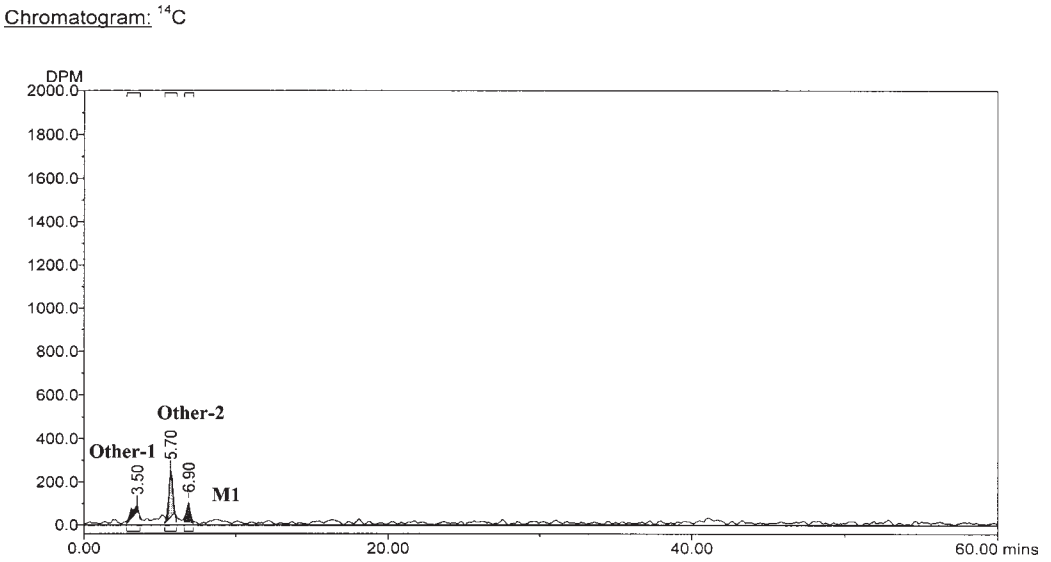

Figure 4. Representative chromatogram of [carbonyl-^{14}C]pyribenzoxim in foliage by radio-HPLC analysis at 7 days after treatment.

level of TRRs from 30 DAT to harvest. At harvest, almost half of TRRs were contained in husk. TRRs in foliage/rest of plant/straw decreased to <0.01% of the application values by harvest, primarily through growth dilution. These low residues in mature plants made their further characterization impractical.

P2, [ring-^{14}C(U)]pyribenzoxim, was treated for the detection of metabolite 3 (**M3**), and therefore, sampling dates were not consistent with the case of **P1** treatment. TRRs in foliage at 7 DAT were observed at almost twice the **P1** case; however, a similar level to the **P1** case was reached at harvest.

Distribution and Identification of Radioactive Residues. The distributions of radioactivity for parent and metabolites in surface wash, extracts, and bound residues in foliage/rest of plant/straw of rice are summarized in Tables 4 and 5. The percentage of TRRs in the surface wash of the **P1**-treated sample decreased gradually to reach, at harvest, less than 2% of the applied amount (Figure 3). In addition, pyribenzoxim was 97.52% TRRs at application and not detected from 60 DAT, while metabolite **M1** was founded to be 26.1% at 7 DAT (Table 4). The extractable radioactivity levels increased to >50% TRRs at 30 DAT and then decreased. The concentration of pyribenzoxim in the extracts decreased to <1.0 ppb from 60 DAT and could not be detected at harvest. **M1** and two unknown compounds (other-1 and other-2) were detected at the only 7 DAT in the solvent extract of foliage, accounting for 3.5% TRRs (21.0 ppb), 3.1% TRRs (19.0 ppb), and 9.0% TRRs (54.3 ppb), respectively (Figure 4). Previous studies have shown that **M1** was a major metabolite, resulting from hydrolysis of the N−O bond linkage, through either abiotic or enzymatic mechanisms,[18,19,28] but was not observed in microsomal metabolism using a rice plant.[29] In the case of this study, **M1** must be formed by photolysis because it was observed mainly in the fraction of surface wash. Two unknown compounds in the solvent extract of foliage/rest of plant/straw could not be identified because of low residue levels and difficulties in cleanup.

However, the percentage of TRRs in unextractable residue increased to a maximum level at harvest, suggesting conjugation or bounding of radiolabel carbons with endogenous plant substances.

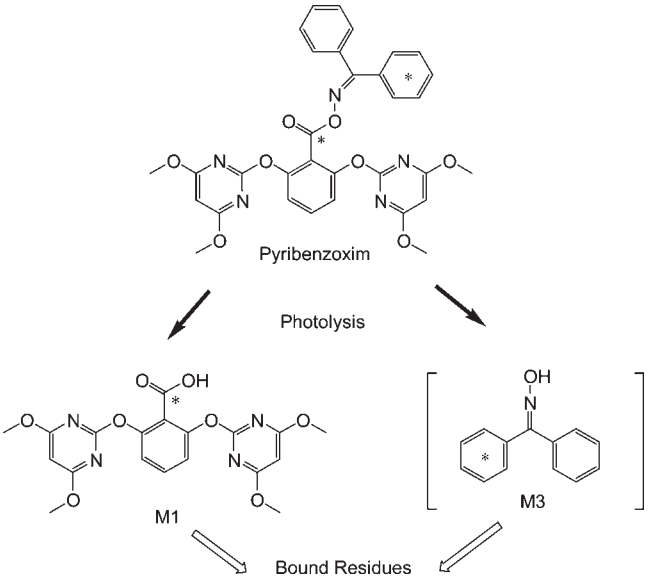

Figure 5. Proposed metabolic pathway of pyribenzoxim in a rice plant.

The surface, extractable, and unextracted residue concentrations in these container trial plants could be higher than in field-grown plants because they were not affected by any rain wash.

The distribution of radioactivity treated with **P2** showed a similar pattern to the treatment of **P1**, giving the highest portion of the unextractable residue level at harvest (Table 5). Pyribenzoxim in the surface wash and extracts was not detected from 60 DAT. Unfortunately, **M3** and other metabolites were not detected even at 7 DAT in the surface wash and extracts.

In conclusion, TRRs decreased rapidly during the growth period (total 120 days) to <17 ppb from 23 876 ppb after [carbonyl-^{14}C]pyribenzoxim was treated at application rates of 50 g of active ingredient (ai)/ha on a rice plant. At harvest, almost half of radioactivity was contained in husk. Therefore, the concentration of final residues in rice was very low, indicating that accumulation of residues will be minimal. When considering the low toxicity of pyribenzoxim to animals, it is unlikely to

present a residue hazard in the environment and food. Metabolite **M1** was observed and identified only at 7 DAT, occupying 26.1% of the solvent washing TRRs and 3.5% of the solvent extract. Expected metabolite **M3** was not detected, even though [ring-^{14}C(U)]pyribenzoxim was used. On the basis of these results, the metabolic pathway of pyribenzoxim in a rice plant was proposed, as shown in Figure 5. The acid metabolite (**M1**) would be formed by the photolysis reaction of the cleavage of the N−O bond linkage of [carbonyl-^{14}C]pyribenzoxim. This compound is known as a major metabolite of pyribenzoxim in rats through either *in vitro* or *in vivo* metabolism.[28,29]

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Funding Sources

This work was supported by grants from the Korea Ministry of Science and Technology and the Korea Food and Drug Administration.

ACKNOWLEDGMENT

The authors thank LG Chem Investment, Korea, for providing chemicals.

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