

## Aerobic Soil Metabolism of a New Herbicide, LGC-42153

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To elucidate the fate of a new sulfonylurea herbicide, LGC-42153 [*N*-((4,6-dimethoxypyrimidin-2-yl)-aminocarbonyl)-2-(1-methoxyacetoxy-2-fluoropropyl)-3-pyridinesulfonamide], in soil, an aerobic soil metabolism study was carried out for 120 days with [<sup>14</sup>C]LGC-42153 applied to a loamy soil. The material balance ranged from 90.7 to 101.5% of applied herbicide. The half-life of [<sup>14</sup>C]LGC-42153 was calculated to be approximately 9.0 days. The degradation products resulted from the cleavage of the sulfonylurea bridge. The metabolites identified during the study were *N*-((4,6-dimethoxypyrimidin-2-yl)aminocarbonyl)-2-(1-hydroxy-2-fluoropropyl)-3-pyridinesulfonamide, 2-(1-hydroxy-2-fluoropropyl)-3-pyridinesulfonamide, and 4,6-dimethoxy-2-aminopyrimidine. No significant volatile products or [<sup>14</sup>C]carbon dioxide was observed during the study. Nonextractable <sup>14</sup>C-residue reached 14.4–30.5% of applied material at 120 days after treatment, and radioactivity was distributed mostly in the humin and fulvic acid fractions.

**KEYWORDS:** LGC-42153; herbicide; sulfonylurea; metabolism; soil

### INTRODUCTION

LGC-42153 [*N*-((4,6-dimethoxypyrimidin-2-yl)aminocarbonyl)-2-(1-methoxyacetoxy-2-fluoropropyl)-3-pyridinesulfonamide] is a new herbicide developed by LG Chem Investment, Korea, and was found to be very effective against annual and perennial paddy weeds with good rice plant selectivity (1). This chemical is a sulfonylurea herbicide, others of which include chlorsulfuron (2), flupyr-sulfuron-methyl (3), imazosulfuron (4), metsulfuron-methyl (5), and rimsulfuron (6). These chemicals are known to inhibit acetolactate synthase in the biosynthetic pathway of the branch-chain amino acids, valine, leucine, and isoleucine, as do other ALS-inhibitors such as imidazolinones, pyrimidinylbenzoates, triazolopyrimidine sulfonamide, and sulfonylamino carbonyl triazolinone herbicides (7, 8). Similarly to other sulfonylureas (2, 9, 10), LGC-42153 is a weakly acidic compound ( $pK_a = 3.5$ ) which exists primarily as a negatively charged species at pH values of the soil solution found in normal agronomic soils. It does not mix well with water (solubility, 114 mg/L at pH 7.0 and 25 °C) and has low vapor pressure ( $<1.86 \times 10^{-5}$  Pa at 25 °C). It has a very low acute toxicity (rat, oral) of  $>5000$  mg/kg, and other toxicology studies are currently underway.

A certain fraction of applied pesticide reaches the soil through a variety of mechanisms. Even when pesticide is applied to crops, some can reach the soil directly through spray drift or

fumigation and indirectly through wash-off. A variety of transformed products can subsequently be formed through biotic or abiotic reactions. Pesticide movement and distribution in soils has received increased attention during recent years because of concern over potential effects on surface and groundwater quality. Soil metabolism studies of pesticides are very important for predicting the degradation behavior of the parent pesticide, for determining the nature and extent of the metabolites formed, for developing soil residue analysis methods, and for assessing the potential environmental hazards.

Extensive research has been carried out on the metabolism of sulfonylurea herbicides in both laboratory and field studies (2, 5). The sulfonylurea herbicides in soils were subjected to both chemical hydrolysis and microbial degradation, and the degradation rate depends on pH, soil moisture content, and soil properties (4, 5). No previous soil metabolism studies of LGC-42153 have been reported. This paper deals with the soil metabolism of LGC-42153 under aerobic conditions, describing the material balance, the degradation pattern of LGC-42153, and the formation of metabolites.

### MATERIALS AND METHODS

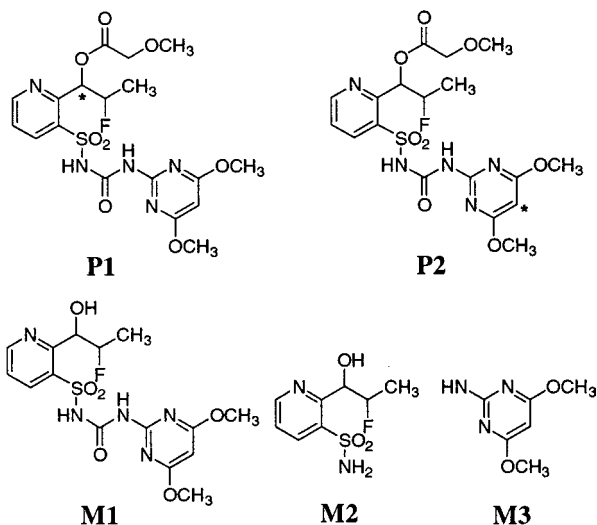
**Chemicals.** The radiolabeled test compounds [propyl-<sup>14</sup>C]LGC-42153 (**P1**) and [pyrimidine-<sup>14</sup>C]LGC-42153 (**P2**), nonlabeled LGC-42153, and metabolites, *N*-((4,6-dimethoxypyrimidin-2-yl)aminocarbonyl)-2-(1-hydroxy-2-fluoropropyl)-3-pyridinesulfonamide (**M1**), 2-(1-hydroxy-2-fluoropropyl)-3-pyridinesulfonamide (**M2**), and 4,6-dimethoxy-2-aminopyrimidine (**M3**), were kindly provided by LG Chem Investment, Daejeon, Korea (**Figure 1**). The radiochemical purities were  $>97\%$ , and specific activities were 2073.48 (**P1**) and 1783.4 MBq/mmol (**P2**). Both compounds were used without further purification. HPLC-grade acetone,

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**Figure 1.** Structures of [propyl-<sup>14</sup>C]LGC-42153 (**P1**), [pyrimidine-<sup>14</sup>C]LGC-42153 (**P2**), and metabolites (**M1**, **M2**, and **M3**). The site of the <sup>14</sup>C label is marked with an asterisk.

**Table 1.** Physicochemical Properties of the Test Soil

pH <sup>a</sup>	6.8	silt (%) <sup>c</sup>	42
organic matter (%) <sup>b</sup>	3.45	clay (%) <sup>c</sup>	12
CEC (mequiv/100 g)	9.8	texture (USDA)	loam
sand (%) <sup>c</sup>	46		

<sup>a</sup> Measured in 1:5 soil–deionized water suspension (17). <sup>b</sup> Walkley–Black colorimetric determination (12). <sup>c</sup> Particle size analysis by the hydrometer method (13).

hexane, ethyl acetate, and methanol were purchased from Duksan Co. (Ansan, Korea). All the other reagents and common chemicals were analytical grade and commercially available.

**Test Soil.** Loamy soil was obtained from Seoul National University by sampling drained paddy soil to a depth of 10 cm. After air-drying at room temperature for 24 h, soil was sieved to remove any particles larger than 2 mm prior to being characterized (Table 1).

**Radioassay.** The radioactivity of all liquid samples was quantitated with a liquid scintillation counter (LSC, Packard TRI-Carb 2100, Boston, MA). Insta-gel XF scintillation cocktail (10 mL) was used for the aqueous or water samples, and Ultima Gold (10 mL) was used for organic samples. The solvent nonextractable soil residue (200–300 mg) was combusted by sample oxidizer (Packard model 307, Boston, MA) after mixing with Combustaid (100–200  $\mu$ L). The [<sup>14</sup>C]carbon dioxide produced was absorbed in Carbo-sorb E (10 mL) and mixed with Permafluor E<sup>++</sup> scintillation cocktail (10 mL) for LSC counting. A Phosphorimager SI system (Molecular Dynamics, Sunnyvale, CA) was used to locate the radioactive spots on TLC plates.

**Chromatography.** Thin-layer chromatography (TLC) was carried out with precoated glass plates (silica gel 60 F<sub>254</sub>, 20 × 20 cm, 0.25 mm thickness, Merck, Darmstadt, Germany) and developed in benzene/ethyl acetate/methanol (15:9:1, v/v). Radioactive spots on TLC plates were detected through co-chromatography with authentic standards, by detection under ultraviolet light (Spectroliner model ENF-260C, Westbury, NY), and by autoradiography using a phosphor image analyzer (Fujix BAS2000, Tokyo, Japan).

Determination of radioactivity and identification of LGC-42153 and metabolites were performed using radioisotope high-performance liquid chromatography (RHPLC) by co-chromatography with authentic compounds. RHPLC was performed using a Waters 510 HPLC system (Waters, Milford, MA) with a Capcell pak C<sub>18</sub> column (4.6 × 250 mm, 5  $\mu$ m, Shiseido, Tokyo, Japan). The column was eluted with a mobile phase containing 40% acetonitrile in water containing 0.1 M acetic acid and 0.02 M ammonium acetate for 20 min, using a flow rate of 1.0 mL/min. UV detection (254 nm) was performed with a variable-wavelength detector. Under these conditions, the retention times for

LGC-42153, **M1**, **M2**, and **M3** were 12.2, 7.4, 4.5, and 3.1 min, respectively. Radioactivity monitoring (Packard Flo-one A 500 radioactivity monitor, Boston, MA, 1.0 mL/min liquid cell) was performed using scintillation cocktail (Flo-Scint III, 2 mL/min).

**Extraction Efficiency.** Samples of soil (50 g, air-dry weight) were adjusted to 75% field moisture holding capacity (14) and treated with 100  $\mu$ L of a 0.04 mg/kg solution of **P1** and **P2** in acetonitrile (11.1 kBq each). Following treatment, the soil was mixed thoroughly by stirring by hand. After 30 min, samples of treated soil were extracted twice each with acetone/water (7:3, v/v, 100 mL), acetone/water (1:1, v/v, 100 mL), and acetone/water/36% HCl (35:35:2, v/v, 100 mL) and subsequently filtered. Triplicate aliquots (1 mL) of soil extracts were analyzed by LSC to measure the radioactivity.

**Soil Incubation.** The soil (50 g, air-dry weight) was weighed into Pyrex glass flasks (100 mL), and an appropriate volume of distilled water was added to adjust the moisture content of the soil to approximately 75% of field moisture holding capacity (14). All soil samples were maintained in a soil metabolism chamber and were preincubated at 25 ± 1 °C for 2 weeks in the dark prior to treatment. Air was passed through a 0.1 M aqueous sodium hydroxide solution containing phenolphthalein indicators (to indicate carbon dioxide saturation of traps) to remove carbon dioxide and was continuously purged through the flasks at a flow rate of 5 mL/min. The **P1** and **P2** treatment solutions were applied in the same manner as for the extraction efficiency samples. Treated soil samples were then incubated again for up to 120 days at 25 ± 1 °C.

The effluent gas from the chamber was passed through two ethylene glycol traps (50 mL) and two potassium hydroxide solution traps (1 M, 40 mL) in sequence to collect volatile compounds and [<sup>14</sup>C]carbon dioxide, respectively, which potentially evolved from the soil. The treated soil was sampled for soil extraction of the chemicals at 0, 1, 3, 7, 15, 30, 60, and 120 days after treatment (DAT). Readjustment of soil moisture content and sampling of [<sup>14</sup>C]carbon dioxide and traps for volatile products were carried out once a week. Triplicate aliquots (1 mL) of ethylene glycol and potassium hydroxide solution from traps were counted by LSC for total radioactivity. Levels of [<sup>14</sup>C]carbon dioxide were confirmed by reacting the potassium hydroxide solution (10 mL) of 120 DAT sample with barium chloride solution (1 M, 10 mL). The resulting supernatant was counted after filtering the barium [<sup>14</sup>C]carbonate precipitate.

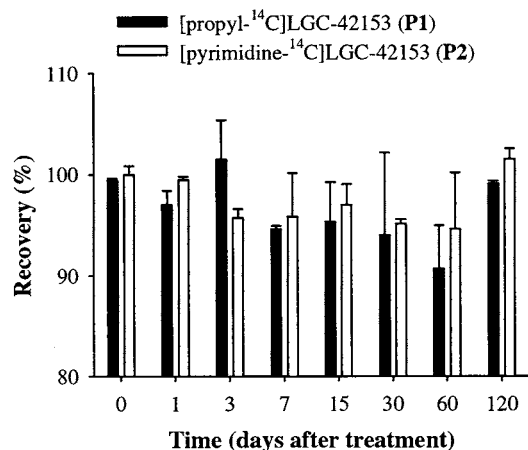
**Extraction and Analysis of Soil.** At each soil sampling date described above, two flasks per treatment (one treatment each of **P1** and **P2**) were taken, and soil samples were extracted with acetone/water (7:3, v/v) and analyzed as described above. After acetone was removed under reduced pressure (40 °C), saturated sodium chloride solution (20 mL) and hydrochloric acid solution (2 M, 20 mL) were added to the aqueous solution, which was then extracted twice with dichloromethane (100 mL). The dichloromethane solution was concentrated in vacuo (40 °C) and dissolved in acetone (2 mL), and the aliquot was analyzed by RHPLC and TLC to determine the concentrations of the parent compound and its metabolites.

After extraction with the acetone/water mixture (7:3, v/v), the remaining soil was then sequentially extracted with acetone/water (1:1, v/v, 100 mL) and acetone/water/36% HCl (35:35:2, v/v, 100 mL) by sonication for 30 min. In each case, the same procedure was repeated twice, and analysis was done by LSC, RHPLC, and TLC in the same manner as described above. All the postextracted soil samples were air-dried in a laboratory hood. When dry, the samples were homogenized and weighed. Triplicate portions were combusted with the oxidizer before LSC counting.

**Distribution of Solvent Nonextractable Radioactivity in Soil.** Soil-bound residues were fractionated with strong base and acid into three fractions: humin, humic acid, and fulvic acid (15, 16). Two-gram aliquots (dry weight equivalents) of nonextractable soil residue were extracted with sodium hydroxide solution (0.1 M, 5 mL), and the supernatant (fulvic and humic acid fraction) was decanted after the extract was centrifuged at 3250g for 10 min. This procedure was repeated until the radioactivity of the extract reached background, and the extracts were combined. Triplicate subsamples (250 mg) of the precipitate (humin fraction) were combusted to determine the content of radioactivity. The extracts were combined, and concentrated

Table 2. Extractability of [ $^{14}\text{C}$ ]LGC-42153 with Different Solvent Systems

compound	applied radioactivity (%)			total
	acetone/water (7:3)	acetone/water (1:1)	acetone/water/36% HCl (35:35:2)	
[propyl- $^{14}\text{C}$ ]LGC-42153 (P1)	94.0	5.31	0.18	99.5
[pyrimidine- $^{14}\text{C}$ ]LGC-42153 (P2)	94.0	2.93	0	96.9

Figure 2. Material balance of soil metabolism of [propyl- $^{14}\text{C}$ ]LGC-42153 and [pyrimidine- $^{14}\text{C}$ ]LGC-42153 under aerobic conditions.

hydrochloric acid was added to adjust the pH to 1. This mixture was extracted and centrifuged, the supernatant was decanted, and the resulting precipitate (humic acid fraction) was washed with hydrochloric acid (0.2 M, 5 mL). After centrifugation, the supernatants (fulvic acid fraction) were combined, and the humic acid precipitate was redissolved in sodium hydroxide (0.1 M, 5 mL) before radiocounting an aliquot (1.0 mL).

**Calculation of Half-Life in Soil Metabolism Study.** Pseudo-first-order kinetics were assumed in order to allow calculation of half-life values. Data were subjected to linear regression analysis (ln of mean % residual LGC-42153 versus time) using SigmaPlot 4.0 software (SPSS Science, Chicago, IL).

## RESULTS AND DISCUSSION

**Extraction Efficiency.** [ $^{14}\text{C}$ ]LGC-42153 was recovered from soil with a high yield ( $\sim 94\%$ ) using acetone/water (7:3, v/v) extraction. A small amount (2.9–5.3%) was detected in the acetone/water (1:1, v/v) fraction (Table 2). The small relative

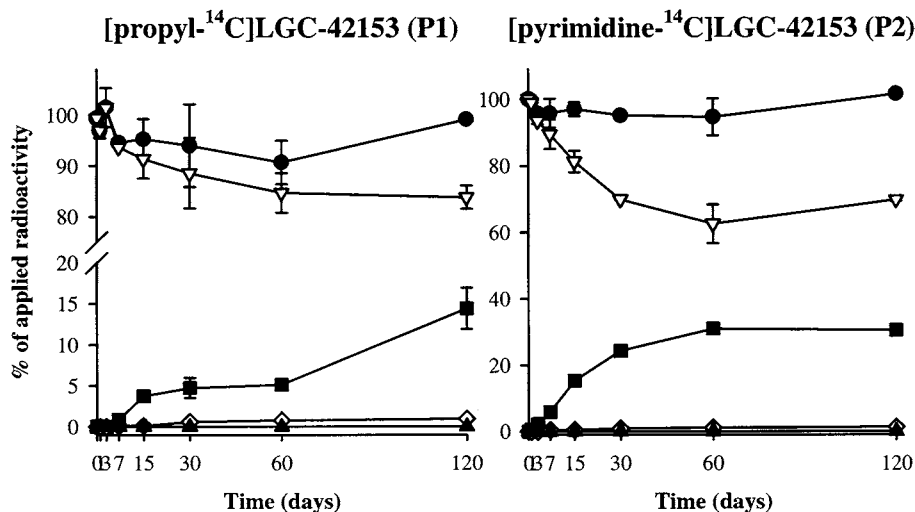
standard deviation values ( $<10\%$ ) confirmed good reproducibility for the method. Although most of radioactivity was recovered in the acetone/water fraction, acetone/water/36% HCl solutions were used to extract metabolites, because they were potentially more polar or acidic than the parent compound.

**Material Balance.** The average material balance over the course of the study was 90.7–101.5% of applied radioactivity for P1 and P2 (Figure 2). Solvent-extractable radioactivity levels decreased gradually with time and accounted for 84% and 70% of the applied radioactivity of P1 and P2, respectively, at 120 DAT. The nonextractable radioactivity levels steadily increased as the soil aged, accounting for 14% of P1 and 30% of P2 at 120 DAT (Figure 3), suggesting that binding of LGC-42153 or degradation products to soil had occurred. The different rate of formation of soil-bound residues suggests that the pyrimidine moiety may be more susceptible to incorporation into the soil matrix than the propyl moiety.

The amount of [ $^{14}\text{C}$ ]carbon dioxide evolved was approximately 0.95–1.28% of the applied radioactivity during the 120-day incubation period, and no other volatile products were detected. These results suggest that the mineralization of LGC-42153 by soil microbes was minimal under aerobic conditions.

To determine the distribution of radioactivity in the nonextractable soil residue, further fractionation of the residue into humin, humic acid, and fulvic acid was performed. Of the radioactivity remaining in the soil at 120 DAT, 7.1%, 7.3%, and 0% of applied radioactivity for P1 were found in fulvic acid, humin, and humic acid fractions, respectively (Figure 4). For P2, 8.6%, 19.7%, and 2.2% of applied radioactivity were found in fulvic acid, humin, and humic acid fractions, respectively. The results indicated that bound radioactivity was associated mainly with humin and fulvic acid fractions.

**Degradation of P1 and P2 and Identification of Metabolites.** Using RHPLC, parent compounds P1 and P2 as well as metabolites M1, M2, and M3 were separated without difficulty.

Figure 3. Distribution of radioactivity in the soils treated with [propyl- $^{14}\text{C}$ ]LGC-42153 (●, total; ▽, extractable; ■, nonextractable; ◇, [ $^{14}\text{C}$ ]carbon dioxide; ▲, volatiles).

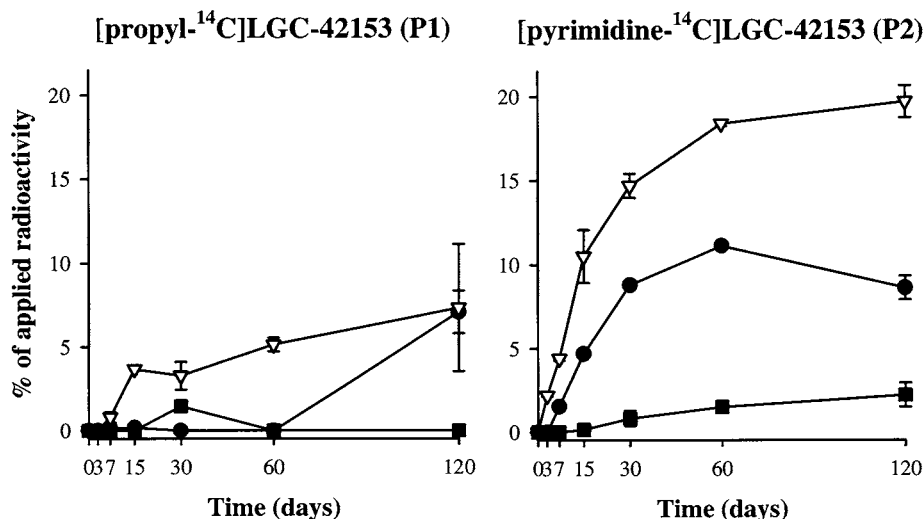


Figure 4. Distribution of solvent-nonextractable radioactivity in the soils treated with [propyl- $^{14}\text{C}$ ]- and [pyrimidine- $^{14}\text{C}$ ]LGC-42153 (●, fulvic acid; ▽, humin; ■, humic acid).

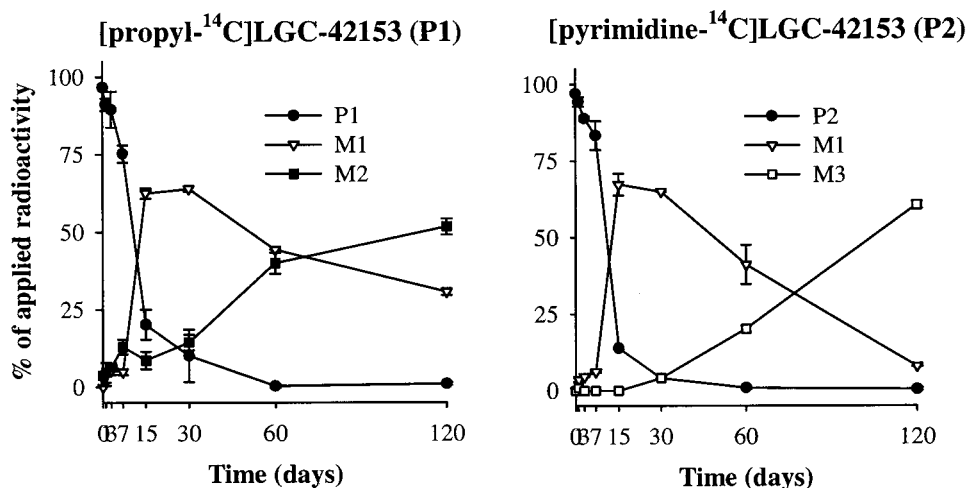


Figure 5. Degradation of [propyl- $^{14}\text{C}$ ]- and [pyrimidine- $^{14}\text{C}$ ]LGC-42153 and formation of metabolites under aerobic conditions.

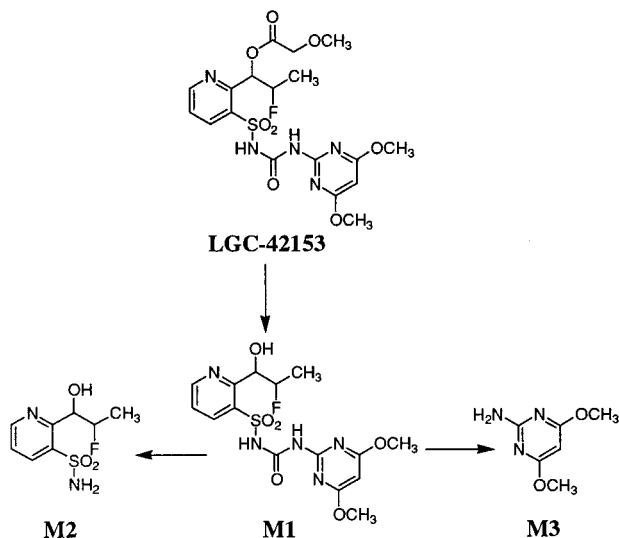
**P1** and **P2** degraded rapidly in aerobic soil, with 1.1 and 0.5% remaining, respectively, at 120 DAT. **M1** from **P1** reached maximum concentration (64.1% of applied radioactivity) at 30 DAT, while **M2** gave maximum concentration (47.8% of applied radioactivity) at 120 DAT (Figure 5). Another metabolite, **M3**, was observed from **P2** with a maximum concentration at 120 DAT (60.6% of applied radioactivity), while **M1** was formed at 8.0%. The estimated half-lives ( $T_{1/2}$ ) of **P1** and **P2** were calculated as 9.4 and 8.7 days, respectively. This short half-life of LGC-42153 in soil (average 9.0 days), compared to those of other sulfonylureas such as amidosulfuron and imazosulfuron (Table 3), may suggest that its structure is susceptible to microbial and chemical degradation. LGC-42153 may be similar to thifensulfuron-methyl (17) and metsulfuron-methyl (5), in which the carboxyester moiety degrades rapidly in soil under aerobic conditions (half-life 2.5–11 days).

On the basis of these results, the metabolic pathway of LGC-42153 is proposed as in Figure 6. **M1** forms as a degradation product by cleavage of the ester bond. Cleavage of the sulfonylurea bridge of **M1**, which has been commonly found in other sulfonylurea herbicides (4–6, 21), results in the formation of metabolites **M2** and **M3**. Sulfonylurea bridge cleavage occurs rapidly in acidic aqueous solution but is slow at neutral to alkaline pH. However, it appears likely that biotic deesterification is the first step, due to the lack of formation of

Table 3. Degradation Rate of Several Sulfonylurea Herbicides in Soil under Aerobic Conditions

pesticide	half-life (days)	soil pH	ref
amidosulfuron	63–246	7.5	18
chlorimuron ethyl	11–17	5.05	19
chlorsulfuron	20	6.4	2
flazasulfuron	13–16		20
imazosulfuron	70	4.5	21
metsulfuron-methyl	9–11	5.2	5
rimsulfuron	24.5	6.5	8
thifensulfuron-methyl	2.5	6.6	17
triasulfuron	33	5.8	22
tribenuron-methyl	12	7.5	23

2-(1-methoxyacetoxy-2-fluoropropyl)-3-pyridinesulfonamide. Once this has occurred, then bridge cleavage takes place. The hydrolysis reaction involves attack of a water molecule on the carbonyl carbon of the sulfonylurea linkage, producing carbon dioxide, the aryl sulfonamide, and amino heterocyclic portions of the parent molecule. In our study, careful examination of the formation and degradation pattern of **M1** compared to those of **M2** or **M3** suggested that **M1** further degrades via cleavage of the sulfonylurea linkage to form **M2** and **M3**. A similar degradation profile was also reported for other sulfonylurea herbicides, including pyrazosulfuron-ethyl and thifensulfuron-methyl (23). The carboxylic acid metabolites of sulfonylurea



**Figure 6.** Proposed metabolic pathway of LGC-42153 in soil under aerobic conditions.

herbicides are inactive against ALS (23). Brown et al. (24) also reported that the crop selectivity of thifensulfuron-methyl is attributed to its rapid detoxification in tolerant plants via methyl ester hydrolysis to yield thifensulfuron, which is herbicidally inactive. The general degradation pathway of sulfonyleureas, such as *O*-dealkylation (4, 5, 21, 25), was not observed in this study due to the short half-life of the parent molecule. To understand more about the fate of LGC-42153, soil metabolism under flooded conditions could be performed as a complementary study.

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