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## BEHAVIOUR OF THE HERBICIDE QUINCLORAC IN A RICE PLANT-GROWN LYSIMETER

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In order to elucidate the environmental behaviour of the herbicide quinclorac (3,7-dichloroquinoline-8-carboxylic acid) applied to rice paddies, [ $^{14}\text{C}$ ]quinclorac (specific activity: 1.50 MBq/mg) was applied at the rate of 300g a.i./ha to a lysimeter (0.564 m ID  $\times$  1 m soil depth) simulating the rice paddy conditions. The  $^{14}\text{C}$ -labeled herbicide was applied 22 days after transplanting and the rice plants were grown by the conventional cultivating method for 88 days until harvest.

The amount of  $^{14}\text{CO}_2$  evolved from the surface of the lysimeter soil was 0.71% of the original radioactivity up to the 14th week after the application, while that of volatile  $^{14}\text{C}$  lost from the surface exhibited the background level, suggesting that quinclorac was stable microbiologically and chemically in this condition. No  $^{14}\text{C}$ -activity was detected in the leachates from the lysimeter during that period, indicating a very slow downward movement of the herbicide in the soil. The average  $^{14}\text{C}$ -activities detected in straw, ear without grains, and chaff after harvest amounted to 0.41, 0.10, and 0.19 mg/kg quinclorac equivalents, respectively, whereas that in brown rice grain was 0.15 mg/kg, indicating that it is far less than the maximum residue limits (MRL) of 0.5 mg/kg quinclorac set by Japan. That is, about 95% of the originally applied  $^{14}\text{C}$  remained in the 30-cm layer from the surface after harvest, whereas a very small fraction was distributed in the different parts of rice plants.

**Keywords:** [ $^{14}\text{C}$ ]quinclorac; lysimeter; mineralization; leaching; bioavailability; soil-bound residue

### INTRODUCTION

Even if the ideal pesticide should be safe for non-target organisms and non-persistent in the environment, certain pesticides, but not all, have some undesirable effects, such as the contamination of our environment which is closely

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related to human health. To diminish these adverse effects, it is very important to elucidate the behaviour of the pesticides used for agricultural practices and then to predict their possible hazards. For this purpose, experiments must be carried out under similar conditions to those of the field. Accordingly, lysimeter experiments using  $^{14}\text{C}$ -labeled compounds under field conditions have been spotlighted. This was supported by some reports<sup>[1-3]</sup> that the results obtained from experiments using well-established lysimeters were in good agreement with those from the field experiments with respect to the behaviour of pesticides, such as the uptake, degradation, residue formation, and translocation of pesticides and/or their metabolites in soil. For one thing, Kubiak *et al.*<sup>[1]</sup> reported about the transferability of lysimeter data to the field by using the leaching and degradation behavior of the  $^{14}\text{C}$ -labeled herbicides, metamitron and methabenzthiazuron.

Quinclorac(3,7-dichloroquinoline-8-carboxylic acid) introduced by BASF AG in 1985<sup>[4]</sup> is a broad-spectrum quinolinecarboxylic acid herbicide used for the control of both watergrass (*Echinochloa* spp.) in rice<sup>[5,6]</sup> and certain broadleaf weeds in turf.<sup>[7,8]</sup> This herbicide was marketed in Korea in 1989 and had been used for the control of *Echinochloa* spp. in both direct-seeded and transplanted rice paddies, but its production and use were banned in 1995, because of its high phytotoxicity to the second crops<sup>[9,10]</sup> grown on rice paddies such as tomato and potato. Quinclorac induced rapid chlorosis and necrosis in susceptible grasses.<sup>[11]</sup> Koo *et al.*<sup>[11]</sup> reported that, although this herbicide is effective for the control of both broadleaf and grass weeds, the mode of herbicidal activity is different for the two weed groups. It was also reported that quinclorac induced electrolyte leakage in barnyard grass (susceptible species) but not in rice (tolerant species), suggesting that quinclorac may directly and indirectly alter the cell membrane integrity in susceptible grasses.<sup>[12]</sup> Wang and Crosby<sup>[13]</sup> reported the photodegradation of quinclorac under indoor and outdoor conditions, showing that the initial loss of quinclorac in the field was rapid, due principally to photodegradation. Chism *et al.*<sup>[14]</sup> studied the uptake, translocation, and metabolism of quinclorac in southern crabgrass and Kentucky bluegrass having different sensitivity. Nelsen<sup>[15]</sup> disclosed some data on the environmental fate of quinclorac in a report submitted to EPA. This includes aerobic and anaerobic aquatic metabolism, aquatic field dissipation, confined crop rotation, photolysis in rice paddy soil and water, confined aquatic dissipation, and soil desorption. Lee *et al.*<sup>[16,17]</sup> reported on the effects of the photosensitizers on the degradation of quinclorac in water and soil, the leaching behaviour in soil columns, and bioavailability to rice plants in the micro-ecosystem.

This investigation was conducted to elucidate the behaviour of quinclorac applied to a rice plant-grown lysimeter simulating the rice paddy conditions in Korea and to evaluate the safety of this chemical in our agriculture.

TABLE I Physico-chemical properties of each layer of the soil in the lysimeter

Soil depth (cm)	pH (H <sub>2</sub> O 1:5)	C. E. C. (mmol(+) / kg soil)	Organic matter	Sand	Silt	Clay	Texture
				%			
0-10	6.3	105.3	3.6	25.6	44.0	30.4	CL
10-20	6.9	102.8	2.7	21.1	48.2	30.7	CL
20-30	7.2	90.1	1.9	21.2	46.6	32.2	CL
30-40	7.4	102.4	1.0	20.5	41.4	38.1	CL
40-50	7.1	91.1	0.9	29.7	31.4	38.9	CL
50-60	6.7	76.2	0.7	28.8	40.9	30.3	CL
60-70	6.5	71.5	0.5	37.3	34.1	28.6	L
70-80	6.4	57.3	0.5	35.9	35.2	28.9	L
80-90	6.1	63.4	0.4	41.3	31.2	27.5	L
90-100	5.5	117.2	1.0	35.0	33.9	31.1	CL

## MATERIALS AND METHODS

A cylindrical lysimeter was manufactured with stainless steel of 8 mm in thickness. The surface area of the lysimeter was 0.25 m<sup>2</sup>, the height 1.1 m, and the inner diameter 0.564 m. The undisturbed soil core of 1.0 m depth was obtained by pressing down the lysimeter on rice paddies located at Bokdae-dong, Cheong Ju, Korea, with the aid of a fork-crane. The physico-chemical properties of the lysimeter soil layers are presented in Table I.

Prior to transplanting, the lysimeter soil was fertilized with N-P-K at the ratio of 150-90-110 kg/ha, 80% of the total nitrogen fertilizer being applied at the beginning and the rest 20% applied at the earing stage. The 35-day-grown rice plant seedlings (*Oryza sativa* cv. Akibare, Japan) were transplanted onto the lysimeter soil on June 2, 1995. Nine hills were transplanted on the lysimeter soil with 3 seedlings/hill. Throughout the cultivation period of 88 days, the soil was flooded to simulate the rice paddies. The average ambient and soil (10-cm depth) temperatures during that period (July, August, and September, 1995) were 24 and 26°C, respectively.

[3-<sup>14</sup>C]Quinclorac (specific activity, 1.50 MBq/mg; purity, >97.6%, confirmed by autoradiography) was supplied by BASF Corporation, Limburgerhof, Germany (Figure 1). The granular formulation, Pul-Ta® (1% quinclorac + 10% bentazon + 89% adjuvant) in which the 1% quinclorac component was replaced by [<sup>14</sup>C]quinclorac was applied onto the lysimeter soil 22 days after transplanting. Because the recommended application rate of quinclorac on rice paddies was very low, only the <sup>14</sup>C-labeled compound was applied. The total amount and radioactivity of quinclorac applied were 7.459 mg and 11.15 MBq, respectively. The <sup>14</sup>C-labeled quinclorac dissolved in about 10 ml of methanol and the quinclorac-free mixed formulation were added to about 200 g of soil. After the

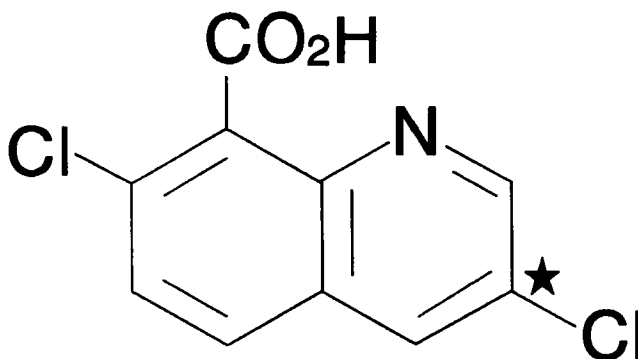


FIGURE 1 Structural formula and labeled position(\*) of quinclorac (3,7-dichloroquinoline-8-carboxylic acid).

methanol was completely evaporated, the soil was scattered over the wet lysimeter soil surface to a rate of 0.3 kg a.i./ha, according to the conventional method in Korea. During the cultivation period, leachates from the lysimeter were collected in a 2L plastic container and the volume and radioactivity measured weekly. Meanwhile,  $^{14}\text{CO}_2$  and volatile substances evolved from the [ $^{14}\text{C}$ ]quinclorac-treated soil surface were trapped by the method described by Lee *et al.*[18] and the radioactivities measured biweekly. That is, the special device for this purpose was a Pyrex<sup>R</sup> glass cylinder (8 cm in inner diameter, 25 cm in height, and the closed top with an inlet and an outlet). Through the inlet,  $\text{CO}_2$ -free air (about 1.2–1.5 ml/min) was introduced to expel the evolved  $^{14}\text{CO}_2$  and volatile substances through the outlet. In a lysimeter, four of them were placed on the surface of the soil. Each device covered an area of 50.24 cm<sup>2</sup> corresponding to about 1/50 of the lysimeter surface.  $^{14}\text{CO}_2$  and volatile substances were trapped in 1N NaOH and 0.1N H<sub>2</sub>SO<sub>4</sub>, respectively.

After harvest, a soil sample from each 5-cm layer was collected down to the 30-cm depth using a soil core sampler attached to a stainless steel core of 5.05 cm diameter and 100 cm<sup>3</sup> volume. The samples were taken randomly from 3 spots and those of each layer were combined together, air-dried, and ground in a mortar for analysis. For partition of the  $^{14}\text{C}$  radioactivity in the methanol extracts from each soil layer between aqueous and organic phases, five milliliters of methanol extracts of soil were evaporated by a bubbling air stream. Five milliliters of distilled water were added, acidified down to pH 2 with three drops of 6N HCl, and mixed homogeneously. Five milliliters of dichloromethane were then added and shaken vigorously with the aqueous solution. The radioactivities in the organic and aqueous phases were measured. The rice plants harvested were separated into straw, ears without rice grain, chaff, and brown rice grain,

freeze-dried (Chem Lab. Instruments LTD., SB4, England), and pulverized with a cutting mill for the measurements of the radioactivity by combustion. Brown rice grain was obtained by removing chaffs. Each 100 g of air-dried soil collected from every 5-cm layer of the lysimeter was shaken with 150 ml of methanol for 4 h and then centrifuged at 27,000g for 10 min to collect the supernatant. This procedure was repeated until the radioactivity of the extract was diminished to the background level. All the extracts were combined and evaporated for further analysis. The exhaustively extracted soil was then burned to measure the non-extractable residues.

To confirm the radiochemical purity of [3-<sup>14</sup>C]quinclorac and identification of its degradation products present in soil extracts, TLC followed by autoradiography was performed. The film was the Fuji X-ray film, Medical(Fuji Photo Film Co., Ltd.). The developing solution was the X-ray film developer (Poohung Photochemical Co., Ltd.). X-FIX (for X-ray film, Poohung Photochemical) was used as the fixer. For TLC, precoated aluminum plates (Art. 5554, DC-Alufolien, silica gel 60F<sub>254</sub>, 20 × 20 cm, 0.2 mm, E. Merck, Germany) were used. The developing solvent was a mixture of ethyl acetate-methanol-acetic acid(70:30:5, v/v/v). In addition, to identify the extracts of each soil layer, image analysis was done using a phosphorescence imaging system (Bio-Rad Model GS-525, U.S.A.).

For the measurements of radioactivity in samples, 0.3 g of each plant or soil sample was burned with the Biological Oxidizer, OX-400 (R. J. Harvey Instrument Corporation, U.S.A.) to give <sup>14</sup>CO<sub>2</sub> which was absorbed in the <sup>14</sup>C-cocktail (CARBO MAX<sup>™</sup> PLUS LUMAC\*LSC B. V., the Netherlands). The radioactivity was measured using a liquid scintillation counter (LSC, PW 4700, Philips) with automatic quench correction. The flow rate of oxygen and nitrogen in the biological oxidizer was 300 mL/min. The temperatures of the catalyst zone and combustion zone were 700 and 900°C, respectively, and the combustion time was 4 min. The toluene cocktail was used for the samples dissolved in organic solvents that were evaporated before the cocktail addition. The radioactivities of <sup>14</sup>CO<sub>2</sub> absorbed in 1N NaOH and volatile substances absorbed in 0.1N H<sub>2</sub>SO<sub>4</sub> were measured using Aquasol (Du Pont, NEN Research Products, U.S.A.).

To compare the metabolic activity of microorganisms present in the lysimeter soil, before and after the growth of rice plants, the dehydrogenase activity of each soil layer was measured by the methods of Lee *et al.*[18] and Casida[19].

## RESULTS

The total amount of <sup>14</sup>CO<sub>2</sub> evolved from the lysimeter soil surface during the cultivation period of 14 weeks after application of [<sup>14</sup>C]quinclorac was 0.71% of the originally applied radioactivities as can be seen in Figure 2, while vola-

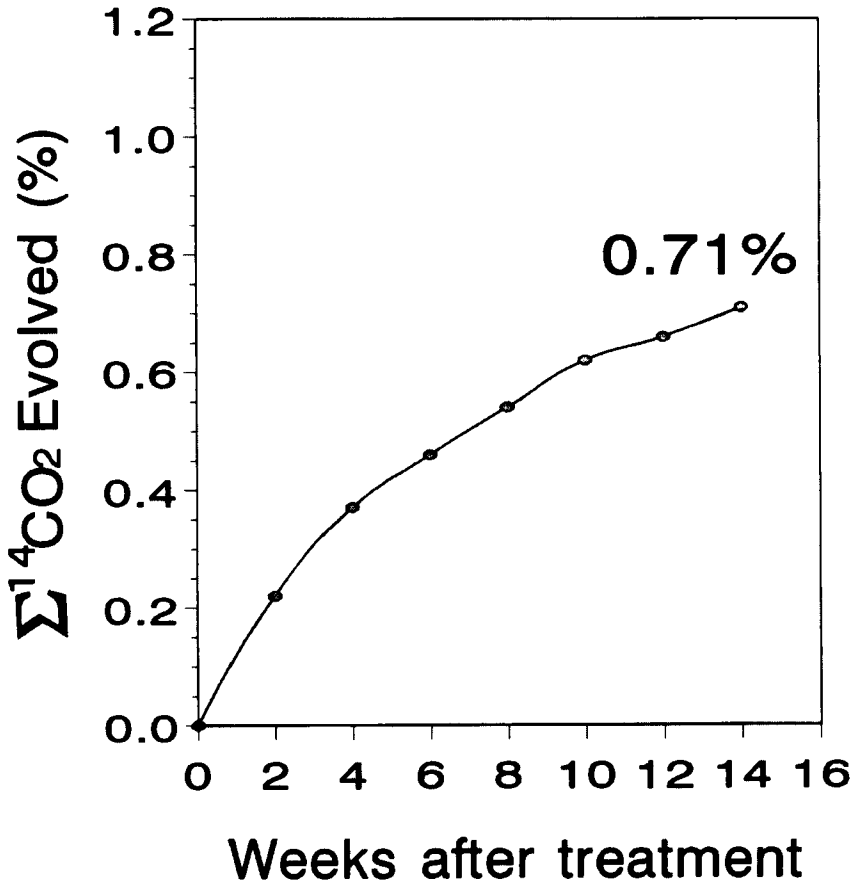


FIGURE 2 Mineralization of [<sup>14</sup>C]quinclorac to <sup>14</sup>CO<sub>2</sub> during the lysimeter experiment of 14 weeks. <sup>14</sup>C-activity applied = 100%.

tilization of the chemical therefrom during that period was in the range of the background level. The amounts of leachates collected during the leaching period of 20 weeks were 15.31L, which corresponds to 61.28 mm precipitation equivalent, but no <sup>14</sup>C-activity was detected in any leachate.

The <sup>14</sup>C-activities distributed in different parts of rice plants after harvest are presented in Table II. <sup>14</sup>C-Activities were detected in all parts such as straw, ear without rice grains, chaff, and brown rice grain. Larger amounts of quinclorac equivalents (0.41 ppm) remained in straw than the other parts. The total <sup>14</sup>C-activity detected in all the parts of rice plants except for the roots was 1.84% of the originally applied <sup>14</sup>C, ranging from 0.07 to 1.45% of the original <sup>14</sup>C in each part.

TABLE II Amounts of [ $^{14}\text{C}$ ]quinclorac equivalents remaining in the different parts of rice plants after harvest

Parts of rice plants	$^{14}\text{C}$ -Radioactivities remaining	
	%	ppm
Straw	1.45 $\pm$ 0.12	0.41 $\pm$ 0.04
Ears without rice grain	0.07 $\pm$ 0.00	0.10 $\pm$ 0.01
Chaff	0.07 $\pm$ 0.01	0.19 $\pm$ 0.02
Brown rice grain	0.25 $\pm$ 0.01	0.15 $\pm$ 0.01
Total	1.84	

Table III presents the distribution of  $^{14}\text{C}$ -activities which were calculated as quinclorac equivalents remaining in each soil layer of the lysimeter after harvest. About 70% of the original  $^{14}\text{C}$  was distributed in the 0–10 cm soil depth from the surface, while the  $^{14}\text{C}$  detected in the 20–30 cm soil layer was less than 7% of the total.

The distribution of  $^{14}\text{C}$ -activity of the methanol extracts from the different layers of the lysimeter soil treated with [ $^{14}\text{C}$ ]quinclorac and collected after the harvest of rice, between aqueous phase and organic phase, is presented in Table IV. As can be seen in this table, most of the  $^{14}\text{C}$ -activity was partitioned into the organic phase, suggesting strongly that quinclorac was not degraded into any polar products in any layer of the lysimeter soil.

Table V shows that quinclorac was tightly bound to soil constituents, such as organic matter and clay minerals to the extent of 66–78% of the residue in the soil, so as not to be extracted with organic solvents.

The image analysis (Figure 3) which was done to identify the  $^{14}\text{C}$ -activities of the extracts of each soil layer, indicates that the extracted compound represents the intact [ $^{14}\text{C}$ ]quinclorac, suggesting the stability of quinclorac in soil. As seen in Figure 3, down to the 20-cm layer of the lysimeter soil, no degradation product was detected.

As can be seen in Table VI summarizing the behaviour of [ $^{14}\text{C}$ ]quinclorac applied to the rice plant-grown lysimeter soil, most of the originally applied  $^{14}\text{C}$

TABLE III Amounts of [ $^{14}\text{C}$ ]quinclorac equivalents remaining in the different soil layers of the lysimeter after harvest

Soil depth from surface (cm)	$^{14}\text{C}$ -Activities remaining	
	%	mg/kg
0–5	42.06 $\pm$ 1.14	0.24 $\pm$ 0.01
5–10	27.38 $\pm$ 0.76	0.15 $\pm$ 0.00
10–15	11.33 $\pm$ 0.51	0.07 $\pm$ 0.00
15–20	7.31 $\pm$ 0.32	0.04 $\pm$ 0.00
20–25	4.50 $\pm$ 0.20	0.03 $\pm$ 0.00
25–30	2.29 $\pm$ 0.22	0.01 $\pm$ 0.00
Total	94.87	



TABLE IV Distribution of  $^{14}\text{C}$ -activity between aqueous phase and organic phase of the methanol extracts from the different layers of the lysimeter soil treated with [ $^{14}\text{C}$ ]quinclorac and collected after harvest. Aqueous phase + Organic phase = 100%

Soil depth from the surface (cm)	Extracted with methanol* (%)	Distribution (%) of $^{14}\text{C}$ after partitioning	
		Aqueous phase	Organic phase ( $\text{CH}_2\text{Cl}_2$ )
0-5	30.80	3.01	96.99
5-10	33.78	1.78	98.22
10-15	25.40	1.75	98.25
15-20	24.74	1.86	98.14
20-25	22.82	2.00	98.00
25-30	25.01	2.23	97.77

\*% of the soil  $^{14}\text{C}$ -activity before extraction.

remained in the soil, especially in the 0-10 cm layer, whereas mineralization, volatilization, leaching, and bioavailability to rice plants were negligible.

Table VII shows the dehydrogenase activities in the lysimeter soil before and after the cultivation of rice plants. Even if the activities increased slightly after the cultivation, the differences were very small.

## DISCUSSION

According to the report of Lee *et al.*[17], the amounts of  $^{14}\text{CO}_2$  evolved from two types of soils with different physico-chemical properties and containing fresh and 60-day-aged residues of quinclorac in the presence and absence of rice plants grown in the microecosystem were all in the range of 0.4-2.2% of the total  $^{14}\text{C}$  applied. In the present investigation, that of  $^{14}\text{CO}_2$  evolved from the surface of the lysimeter soil was also less than 1% of the total  $^{14}\text{C}$ , suggesting that this herbicide is not vulnerable to chemical and microbial degradation in

TABLE V Comparison of  $^{14}\text{C}$ -activities extracted and non-extracted from the lysimeter soil treated with [ $^{14}\text{C}$ ]quinclorac and collected after the harvest of rice grown on it.

Lysimeter soil layer from the surface (cm)	$^{14}\text{C}$ -activities extracted with methanol	Non-extractable bound residues	Recovery
			%
0-5	30.80	70.59	101.39
5-10	33.78	65.54	99.32
10-15	25.40	77.51	102.91
15-20	24.74	74.74	99.48
20-25	22.82	76.01	98.83
25-30	25.01	73.46	98.47

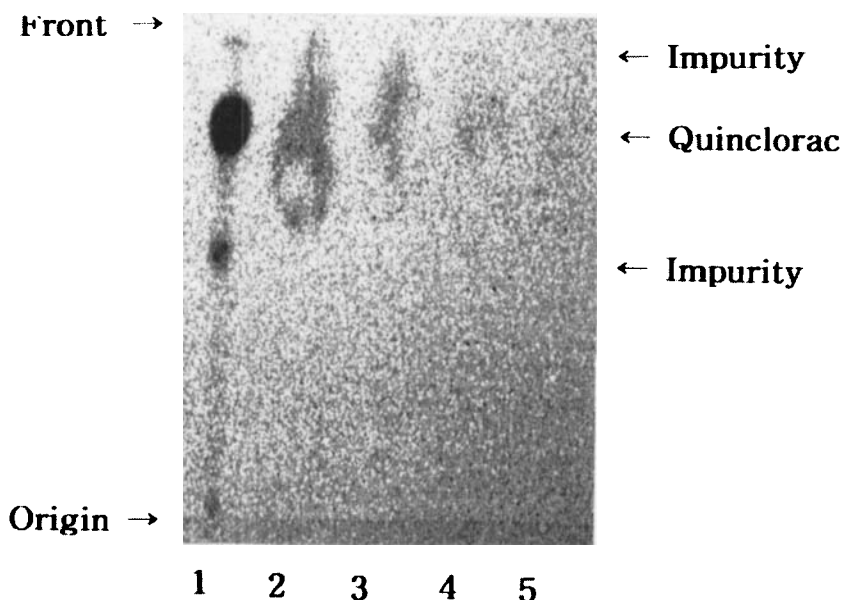


FIGURE 3 Autoradiogram of the methanol extracts from each layer of the lysimeter soil treated with [ $^{14}\text{C}$ ]quinclorac and collected after the harvest of rice plants grown on it. TLC developing solvent: ethyl acetate-methanol-acetic acid (70:30:5, v/v/v). 1: Authentic [ $^{14}\text{C}$ ]quinclorac, 2: 0–5 cm layer, 3: 5–10 cm layer, 4: 10–15 cm layer, 5: 15–20 cm layer

this soil. These results are in good agreement with those of Wang and Crosby<sup>[13]</sup> and the report of Nelsen<sup>[15]</sup>.

Wang and Crosby<sup>[13]</sup> reported that quinclorac dissipates completely within 30 days in the flooded field and that photo-oxidation by natural field-water oxidants plays an important role in the degradation. In plants, quinclorac is systemically translocated to the roots and leaves.<sup>[14–17,20]</sup> Chism *et al.*<sup>[14]</sup> reported that the uptake amounts of foliar-applied [ $^{14}\text{C}$ ]quinclorac in southern crabgrass and Kentucky bluegrass were 85 and 66%, respectively, of the original  $^{14}\text{C}$  within 0.5 h, indicating its rapid translocation. On the contrary, in our investigation where quin-

TABLE VI Distribution of [ $^{14}\text{C}$ ]quinclorac after application onto the rice plant-grown lysimeter soil. Radioactivity applied = 100%

$^{14}\text{CO}_2$ evolved	$^{14}\text{C}$ volatilized	$^{14}\text{C}$ in rice plant (except root)	$^{14}\text{C}$ leached	$^{14}\text{C}$ remaining in soil	Recovery (approx.)
0.71	BG	1.84	BG	94.87	97.42

\* BG: Background

TABLE VII Comparison of the dehydrogenase activities of the lysimeter soil before and after rice plant cultivation.

Substrate	Formazan formation (mg/5g soil*)	
	Before cultivation (10–20 cm soil depth)	After cultivation (15–20 cm soil depth)
None	0.27	0.32
0.05M-Glucose	0.54	0.59
0.1% Yeast extract	0.44	0.52
3.7% Brain heart infusion broth	0.67	0.72

\*Soil weight was on the dry weight basis.

clorac was treated on soil, although  $^{14}\text{C}$ -activity was detected in all the different parts of rice plants, the amounts were very small. The total  $^{14}\text{C}$  translocated to various parts of rice plants except for roots (1.84% of the original  $^{14}\text{C}$ ) was negligible as compared with that remaining in the lysimeter soil (95% of the  $^{14}\text{C}$  applied). Larger amounts of  $^{14}\text{C}$  were detected in straw than the other parts. Especially, its concentration in brown rice grain was less than the maximum residue limits (MRL) of 0.50 ppm set by Japan, suggesting that it would be safe for human beings.

Wang and Crosby<sup>[13]</sup> pointed out in their investigation that low adsorption of quinclorac to sediment ( $K_{oc} < 1$ ) and low Henry's law constant ( $H' 2.3 \times 10^{-8}$ ) ruled out the possible loss of quinclorac from the field test plots by binding or volatilization. Nevertheless, in our investigation (Table V), the non-extractable bound residues amounted to 65.5–77.5%. This result emphasizes that the roots of rice plants (rhizosphere) played an important role in forming the bound residues.

Meanwhile, no  $^{14}\text{C}$ -activity was detected in any of the leachate samples collected from the lysimeter during the leaching period of 20 weeks. Also, after harvest, most of the  $^{14}\text{C}$  applied onto the lysimeter soil surface was distributed in the 0–15 cm layer of the lysimeter, indicating that quinclorac moved downward very slowly and that it was tightly bound to soil materials. The fact that most of the  $^{14}\text{C}$ -activity extracted from each soil layer represented the intact quinclorac indicates strongly its stability in soil. It was reported that quinclorac is only slightly adsorbed on soil. Depending on soil type and organic matter content, it is relatively mobile, this mobility increasing with higher percolation rates in fields.<sup>[20]</sup> In an investigation<sup>[16]</sup> on the leaching behaviour of [ $^{14}\text{C}$ ]quinclorac in soil columns, Lee *et al.* reported that 81.1% of the originally applied  $^{14}\text{C}$  was leached through the soil column, whereas in the presence of rice plants grown on it, the leached  $^{14}\text{C}$  decreased to 36.8%. Another report<sup>[15]</sup> pointed out that quinclorac is hydrolytically stable in the laboratory condition, and although the low  $K_d$  values ( $K_{ds} = 0.05\text{--}0.516$ ) indicate potential high

mobility, the dissipation data in the aquatic field showed little mobility below the 12 inch soil depth, suggesting sensitized aqueous photolysis. It was assumed that the low mobility of quinclorac in our investigation would be due to the field-simulating lysimeter conditions with many factors, the different physico-chemical properties of the lysimeter soil with relatively large amounts of organic matter (organic matter in the 0–10 cm depth: 3.6%), and furthermore the presence of rice plant roots (rhizosphere effect).<sup>[16,21–23]</sup>

Based on the results obtained from this study, it appears that quinclorac poses little risk for the contamination of groundwater and rice grain under our environmental conditions.

### **Acknowledgements**

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