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Environmental Fate of the Herbicide Molinate in a Rice-Paddy-Soil Lysimeter

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Agrochemicals, such as herbicides that are generally applied to agricultural soil are one of the most significant contaminants in the agricultural soil-water system. One of the main mechanisms of water contamination by pesticides is their movement from treated soil to water. Pesticide contamination of the soil-water system is an environmental concern with respect to the effects of pesticides on public health as well as non-target species. Thus, examining the fate of pesticides in the soil-water environment is required for pesticide examiners to predict the pesticide of the soil-water contamination.

Molinate is a thiocarbamate herbicide widely used for weed control in agricultural crops worldwide. In Korea, molinate has been used in several formulation types since 1976. The use of molinate in Korea has sharply increased since the 1990s as labor shortage in the rural community decreased. Molinate contributes to about 30% of the total herbicide used in Korea. Many studies have reported that molinate is found in river-water at variable concentrations (Albani et al 1998; Cerejeira et al 2003; Okamura et al 2002). Some toxicological studies have demonstrated that molinate and its metabolites result in adverse effects such as reproductive and testicular toxicity on mammalians (Ellis et al 1998; Jewell et al 1998; Yan et al 1997). Considering that groundwater is the most important source of drinking water for human consumption in rural areas in Korea, where molinate has been applied to rice fields, predicting the environmental behavior of molinate is important to maintain the quality of water. Although molinate is known to degrade rapidly via volatilization and photodegradation in soils (Konstantinou et al 2001), there is relatively little information in the open literature on the overall fate of molinate under natural conditions. The objective of this study is to examine the fate of molinate in a rice-paddy-soil lysimeter. A combined mixture of molinate and ^{14}C-molinate was applied to a lysimeter simulating rice paddy soil conditions, and the fate of molinate was investigated by measuring the total ^{14}C radioactivity in the collected leachate, evolved CO_2, and ^{14}C-residues in the soil/rice plants.

MATERIALS AND METHODS

Lysimeter was prepared from a stainless steel column (85-cm long x 56.4-cm i.d.,

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8.0-mm in thickness). The column was placed in a rice paddy located at an experimental field. The column was then gently pressed into the paddy field by using a forklift to a depth of 75-cm. The column containing the soil was carefully removed from the paddy site and placed in a PVC column (85-cm long x 110-cm i.d., 5.0-mm in thickness). The space between the lysimeter and PVC column was filled with the paddy soil to simulate field conditions, after which pesticide-free-water was added to the lysimeter to maintain 1-cm of water-depth from the soil surface until the experiment terminated. The lysimeter was then equilibrated in this state for a three-week period. Twenty-seven rice plant seedlings (*Oryza sativa* L.), 15 days old, were transplanted into nine different sites of the lysimeter soil in the first and second year, respectively. The lysimeter soil was fertilized with N-P-K according to a traditional method. The lysimeter experiment was performed under greenhouse conditions for better control of the environmental conditions. Some physical properties of the soil are presented in Table 1.

Table 1. Physicochemical characteristics of lysimeter soil.

Soil depth (cm)	pH (1:5H_2O)	OM (%)	CEC ($cmol^+/kg$)	Soil texture
0-14	5.28	1.65	39.52	Sandy loam
14-25	5.46	1.44	32.45	
25-45	5.96	1.15	23.95	
45-69	6.29	0.90	17.86	
69-75	6.39	0.78	17.27	

^{14}C-molinate (*S*-ethyl hexahydro-[2-^{14}C]-azepine-1-carbothioate, purity 98.5%, sp. act. 980 MBq/mmol) and unlabeled molinate (purity 95.5%) were kindly provided by Zeneca Agrochemicals (England). ^{14}C-molinate (7.44 MBq) and unlabeled molinate were dissolved in 10 ml of methanol and carefully added dropwise to 250 g of paddy soil. The treated soil was then mixed thoroughly and added uniformly to the lysimeter soil eleven days in the first year, after the rice transplantation, which resulted in an initial concentration of 1.5 kg (a.i.)/ha. The leachates from the lysimeter were collected in a brownish 4L-glass bottle placed beneath the lysimeter during a period of rice cultivation in each experimental year. The drainage of the lysimeter was closed during a period from after harvesting the rice plants in the first year to before transplanting the rice plants in the second year. The radioactivity in the leachate was determined bi-weekly in Aquasol (PackardBioscience, USA) by using a Packard Tri-Carb TR 1600 liquid scintillation counter (LSC).

Mineralization of ^{14}C-molinate was investigated by measuring $^{14}CO_2$ from the lysimeter soil. For this, four glass tubes (7.5-cm i.d. x 25-cm long) were placed on the surface of the lysimeter soil and pushed into a soil depth of 20-cm. CO_2-free air was supplied to the glass tubes at a rate of 20 mL/min and then the evolved $^{14}CO_2$ was trapped in a series of 2 *N* NaOH solution traps. Radioactivity was measured bi-weekly in Aquasol (PackardBioscience, USA), as described above.

The lysimeter soil samples were collected at 10-cm vertical increments to a depth of 30-cm in the first year. The samples were collected from three different sites by using a soil core sampler. The sampling sites were filled with molinate-free paddy soil to have the same experimental conditions of the lysimeter soil in the second year. In the second year, the soils were taken from different sites again in 10 cm-increments into a depth of 60-cm, as described above. The samples taken from the same soil depth were combined after air-drying. The lysimeter soil at each soil depth was taken completely and air-dried to measure total soil weight. To measure radioactivity in the lysimeter soil, 0.3-g (air-dry wt basis) of the soil were combusted using the Combusto-Cone™ (Packard BioScience, USA) and trapping the evolved CO_2 in the scintillation fluid, Carbosorb E plus PermaFluor E^+ (PackardBioscience, USA). Radioactivity in the combusted samples was determined by using the LSC. When ^{14}C-molinate was fortified in molinate-free soil samples and then the soils were combusted as described above, more than 95% recovery of radioactivity was observed.

To investigate what residue types of molinate can be found in the lysimeter soil, solvent extractable and bound (solvent non-extractable) residues were characterized. For this, the lysimeter soil obtained from a depth of 10-cm, in which the highest radioactivity was detected, was air-dried. Fifty grams of the air-dried lysimeter soil were extracted with two volumes of methanol. The extraction was repeated until the radioactive level of the final extract reached the radioactive level of the solvent mixture. All extracts were combined and the radioactivity was determined, after which the extracts were regarded as solvent extractable residues of molinate. The radioactivity in the organic extract was determined in Ready Organic ™ (Beckman Co., USA) by using the LSC. The extracted soil was then used for bound residues characterization. The extracted soil was air-dried and combusted to measure total radioactivity, as described above. The air-dried soil was further extracted with two volumes of 0.1 *M* sodium pyrophosphate until no more radioactivities in sodium pyrophosphate extract were detected. The sodium pyrophosphate insoluble fraction was regarded as the humin portion, while the sodium pyrophosphate soluble fraction was further characterized to obtain fulvic and humic acid portions. The sodium pyrophosphate insoluble soil was air-dried and combusted, as above, for determining the radioactivity, as described above, of the humin portion of the lysimeter soil. The sodium pyrophosphate soluble fraction was acidified with 6 *N* HCl until no additional precipitate was observed. The acidified extract was then centrifuged at 10 000 x g for 30 min. The supernatant and precipitate were regarded as the portion of fulvic acid and humic acid, respectively. The total volume of supernatant was measured and an aliquot of the supernatant was used for determining the radioactivity in the portion of fulvic acid of the lysimeter soil. The precipitate was dissolved in 0.1 *N* NaOH and used for determining the radioactivity in the portion of humic acid. The radioactivity in the aqueous extracts was determined in Aquasol (PackardBioscience, USA), as described above. For the radioactivity assay in the rice plants, the rice straw, ear and grain samples were separately obtained from the harvested rice plants. The grain samples were removed from the rice ear and

homogenized with a rice mill (HMW-1800, Hyunju Electronics, Seoul, Korea) and the total sample weight was measured. The rice straw and ear samples were cut into small pieces using scissors. The samples were freeze-dried and pulverized with a mortar and pestle and then their total weights were measured. A portion (0.2 g dry wt) of each sample was combusted and used for determining radioactivity, as described above.

Table 2. ^{14}C radioactivity in the leachates and percolate from lysimeter soil.

Leaching period	% of applied $^{14}C^{*}$	Amount of percolate (L)
First year (1-20 wk)	1.05 ± 0.07	305.46
Second year (1-20 wk)	0.34 ± 0.02	217.45

* Means of three determinations ± SD.

To analyze the degradation products of molinate in the leachates, the leachate samples were extracted with two volumes of ethyl acetate under pH 2 condition. After extraction, the organic phase was dehydrated over anhydrous sodium sulfate and evaporated to dryness in an evaporator. The dried extract was dissolved in methanol and spotted onto TLC plates (silica gel 60 F_{254} 20 x 20 cm, 0.5 mm thickness, Merck, Germany) for autoradiography. The solvent mixture for developing the TLC plate was petroleum ether/ethyl acetate/acetone (5/1/1, v/v/v). The film for autoradiography was a Bio-imaging film (Fuji, Tokyo, Japan), and the autoradiogram for detecting degradation products was a BAS 1500 Bio-imaging analyzer (Fuji, Tokyo, Japan).

The data given in this study are the means of three determinations, unless otherwise stated. The data of three replications are not significantly different at the 7% level of maximum standard deviation by using a Microsoft Excel computer program (Windows® NT).

RESULTS AND DISCUSSION

Table 2 shows the data for amounts of applied ^{14}C in the leachate from the lysimeter soil after ^{14}C-molinate treatment. The radioactive counting levels detected in the leachate were approximately 1.05 and 0.34% of applied ^{14}C in the first and second year, respectively. Higher ^{14}C-radioactivity was observed in the first year as compared to the amount observed in the second year. The radioactivity in the leachate was gradually increased until 4 wk after treatment, but no significant changes in the leaching pattern were observed over the two-year period. The amounts of percolate leached from the lysimeter soil were 305.5 and 217.5 L in the first and second year, respectively. This suggests that compaction of the lysimeter soil was not significant under the experimental condition.

To analyze the degradation products of molinate in the leachates, the leachates

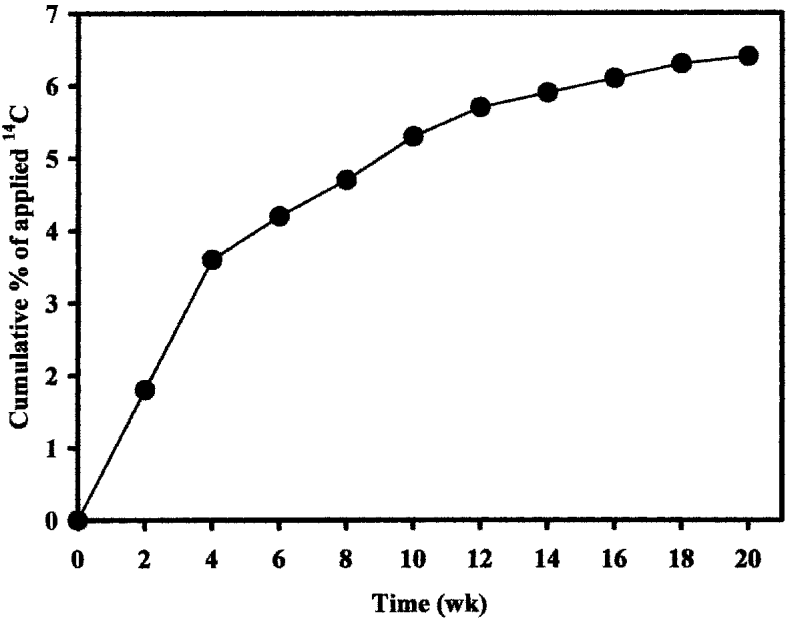

Figure 1. Cumulative percent values of mineralization from lysimeter soil in the first year experiment.

were extracted with organic solvent and developed on TLC plates. Three major degradation products were observed on the TLC plates. One of them showed the same *Rf* value as that of molinate, suggesting molinate leached from the lysimeter soil. Most of the radioactivity in the leachates was due to the degradation products, giving the radioactivity counting level of unchanged molinate less than 10% of the total ^{14}C-radioactivity in the leachates. Molinate was not detected in the leachates collected in the second year. The water solubility of molinate is known to be 990 ppm, suggesting a high potential of leaching to groundwater. A number of studies reported that molinate was detected in the ground and surface waters, the lake and rivers, ranging from 0.03 to 330 ppb (Emmanouil and Euphemia 1999; Okamura et al 2002; Sudo et al 2002). In this study, the concentration of molinate detected in the leachate was estimated to be 1.74 ppb (radioactivity equivalent), suggesting that both molinate and its degradation products should be considered for groundwater contamination.

Figure 1 shows the losses of applied ^{14}C-molinate by mineralization. Molinate mineralization appeared to proceed slowly throughout the experiment. About 7% of applied ^{14}C-molinate was mineralized in the lysimeter soil for the two-year period. More than 99% of the total amount mineralized was observed in the first year. The amount mineralized in the second year was negligible. A possible reason for low mineralization with time could be the strong adsorption of

molinate and its degradation products to soil, which might result in the chemicals being unavailable for the microorganisms to degrade. Based on the experimental data, it was suggested that microbial mineralization was not the main metabolic pathway of molinate fate in the soil.

The data for ^{14}C-radioactivity distributed in the lysimeter soil are shown in Table 3. A significantly high ^{14}C-radioactivity was observed in the surface soil of the lysimeter, giving more than 74% of the total radioactivity detected in the lysimeter soil segments. Less than 1% of applied ^{14}C was detected below 30 cm of lysimeter soil depth. The ^{14}C-radioactivity in the lysimeter soil decreased with increased soil depth and decreased organic matter content. These results suggested that molinate remained mainly in the soil with high organic matter after application. The data for the distribution of ^{14}C-radioactivity in the lysimeter soil showed a good agreement with the data of molinate leaching, since a low concentration of molinate was detected in the leachate, which might be due to the strong adsorption of molinate in the surface soil.

Table 3. Distribution of applied ^{14}C radioactivity in lysimeter soil.

Soil depth (cm)	% of applied $^{14}C^*$	
	First year	Second year
0-10	14.32 ± 0.13	13.27 ± 0.66
10-20	3.20 ± 0.07	2.89 ± 0.12
20-30	1.09 ± 0.03	1.07 ± 0.08
30-40		0.30 ± 0.01
40-50		0.27 ± 0.04
50-60		0.08 ± 0.02

* Means of three determinations ± SD.

Table 4. Percent values of ^{14}C bound residues of molinate in lysimeter soil.

Bound residues	% of applied $^{14}C^*$	
	First year	Second year
Solvent nonextractable	48.21 ± 2.32	90.36 ± 5.27
Humin	2.72 ± 0.46	49.81 ± 2.32
Fulvic acid	44.90 ± 3.74	39.42 ± 1.91
Humic acid	0.38 ± 0.06	1.08 ± 0.05

* Means of three determinations ± SD.

Molinate bound residues in the lysimeter soil were approximately 48.2 and 90.4% of the total radioactivity detected in the soil in the first and second year, respectively (Table 4). Significantly higher bound residues were observed in the second year compared to those in the first year, suggesting the amount of bound residues increased with time. The distribution of ^{14}C bound residues in the humin, fulvic acid, and humic acid fractions of the soil were determined to be about 2.7,

44.9, and 0.4% of the total bound residues in the first year, respectively. The distributions in humin, fulvic acid, and humic acid portions were approximately 49.8, 39.4, and 1.1% of the total bound residues in the second year. The main fraction of ^{14}C in the soil was fulvic acid portion in the first year, but humin and fulvic acid portions in the second year. From these results, it was suggested that the molinate and its metabolite that remained in the soil after application were such bound residue types.

Table 5. Percent values of radioactivity of applied ^{14}C in the rice plants grown in lysimeter soil.

Plant structure	% of applied $^{14}C^*$	
	First year	Second year
Rice straw	11.08 ± 0.07	0.06 ± 0.01
Ear	0.03 ± 0.01	0.01 ± 0.00
Rice grain	0.08 ± 0.01	0.01 ± 0.00

* Means of three determinations ± SD.

After application of ^{14}C-molinate, approximately 11.5% of applied ^{14}C was detected in the rice plants in the first year. This radioactivity counting level was less than 0.1% in the second year (Table 5). More than 96% of the total ^{14}C-radioactivity in the rice plants was detected in the rice straws, giving the lowest radioactivity in the ear (Table 5). The radioactivity in the rice plants was not extractable using organic solvent, suggesting that the radioactivity was mainly due to degradation products of molinate.

A negligible radioactivity in the second year was observed as compared to that in the first year. This might be due to the fact that most of the applied radioactivity remained as the bound residues in the soil, which resulted in the chemicals being unavailable to be taken up by the rice plants.

Mass balance calculated on the basis of the radioactivity detected in the leachates, lysimeter soil, rice plants, and CO_2 was estimated to be about 38.2% of the applied ^{14}C. Molinate is known to be very volatile under rice-paddy conditions (Deul et al 1978; Imai and Kuwatsuka 1988; Soderquist et al 1977). Thus, the remaining 61.8% of the applied ^{14}C was suggested to be the losses by volatilization. Overall, this study suggested that the main pathways for molinate fate in the soil are accumulation in the surface soil and volatilization to the atmosphere. With our limited knowledge, this study is the first to report the overall environmental behaviors of molinate in lysimeter simulating rice-paddy.

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