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A Clomazone Immunoassay To Study the Environmental Fate of the Herbicide in Rice (*Oryza sativa*) Agriculture

Mariana Carlomagno,[†] Cecilia Mathó,[†] Guillermina Cantou,[‡] James R. Sanborn,[§] Jerold A. Last,[∥] Bruce D. Hammock,[§] Alvaro Roel,[‡] David González,[⊥] and Gualberto González-Sapienza*,[†]

[†]Cátedra de Immunología, Instituto de Higiene, DEPBIO, Facultad de Química, UdelarR, 11600 Montevideo, Uruguay, [‡]National Institute of Agricultural Research (INIA), 33000 Treinta y Tres, Uruguay, [§]Department of Entomology and Cancer Research Center and [∥]Pulmonary/Critical Care Medicine, School of Medicine, University of California, Davis, California 95616, and [⊥]Laboratorio de Biocatálisis y Biotransformaciones, DQO, Facultad de Química, UdelaR, CC 1157, 11600 Montevideo, Uruguay

The environmental impact of rice agriculture is poorly studied in developing countries, mainly due to limitations of the analytical capacity. Here, we report the development of a clomazone enzyme-linked immunosorbent assay as a fast and cost-effective tool to monitor the dissipation of this herbicide along the harvest. Antibodies were prepared using different strategies of hapten conjugation, and the best hapten/antibody pair was selected. It proved to be a reliable tool to measure the herbicide in the 2.0−20 ng/mL range in field samples, with excellent correlation with high-performance liquid chromatography results. The assay was used to study the dissipation of the herbicide in the floodwater of experimental rice paddies in Uruguay. Large differences in the residual amounts of herbicide were observed depending on the flooding practices. Because of its robustness and simplicity, the assay may be useful to delineate and monitor management practices that can contribute to minimizing the release of the herbicide in the environment.

KEYWORDS: ELISA; clomazone; rice; HPLC; *Oryza sativa*

INTRODUCTION

Agricultural production is one of the key components of domestic and export economies of developing countries. Such is the case of Uruguay, which, worldwide, is among the top 10 exporters of rice (*Oryza sativa*) and where vast land area is dedicated to this crop. Unlike other regions of the world, the cereal is commonly grown in rotation with pastures. A typical rotation consists of 1 or 2 years of rice, where only one crop per year is grown, followed by 3−4 years of pastures. This alternate use of the land minimizes the build up of pests and diseases and reduces the use of agrochemicals (*1*). This agricultural practice is expected to be more benign to the environment than alternative, more intensive production systems, but the actual impact of this culture methodology is largely unknown. One of the main herbicides used on this crop is clomazone [2-(2-chlorophenyl)-methyl-4,4-dimethyl-3-isoxazolidinone], which is used in 78% of the planted area. Before flooding the fields, clomazone alone or in combination with other herbicides is applied for the control of weeds (*2*). Dissipation studies performed in China and Australia indicated that after flooding, the herbicide starts to be degraded or volatilizes into the atmosphere, and following safe withholding periods, its release into water streams can be prevented (*3*,*4*). This is important, because there are numerous studies revealing that in high doses, clomazone can cause endocrine disruption in fishes or represent a threat to the biota (*5*). In addition, contamination of drainages and rivers holds the potential for unacceptable levels of clomazone in drinking water. The European Economic Community (Directive No. 0/778/EEC) sets a maximum permissible concentration of pesticides in water for human consumption at 0.1 ng/mL (*6*), and the environmental limit required for surface water is less than 3 ng/mL (*7*).

Instrumental analytical methods such as gas chromatography (GC) or high-performance liquid chromatography (HPLC) are the reference techniques for the analysis of pesticides. However, the equipment is expensive to buy and operate and requires highly trained personnel and large amounts of organic solvents. In addition, to attain the sensitivity required to determine the rigorous limits of herbicides in waters, it is necessary to use methods for extraction and preconcentration of the sample, adding costs to the analysis and making sample preparation labor-intensive and tedious (*8*). An alternative to this situation, particularly suitable for low-income countries, is the introduction of methodologies that provide reliable data and are yet cost-effective (*9*). Immunoassays have consolidated as a mature technology, and many immunoassays have been incorporated as official methods by the Environmental Protection Agency of the United States. Immunoassays rely on the high affinity and specificity with which antibodies recognize their target analytes. They attain low detection limits and allow the parallel processing of many samples, and clean up steps are less stringent or unnecessary and are simple, fast, and portable. The most common format is the enzyme-linked immunosorbent

*To whom correspondence should be addressed. Tel: (5982)4874334. E-mail: ggonzal@fq.edu.uy.

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assay (ELISA). While immunoassays do not completely substitute for the conventional instrumental methods, they have enormous potential as screening tools for large-scale and sustainable monitoring (*10*).

The aim of this work was the development of an ELISA for the detection of clomazone, to provide a fast and cost-effective tool to monitor the fate of this herbicide in rice cultivation. Immunoassays for clomazone have been reported previously (*11*), but they did not possess enough sensitivity nor have they been used to study the dissipation of the herbicide in regard to this crop. In the current studies, polyclonal antibodies were prepared using different strategies of hapten conjugation. The best hapten/antibody pair was selected, and buffer conditions were adjusted to minimize matrix effects, enhance recovery, and improve long-term reagent stability. The assay was further compared against HPLC using water samples from rice fields. The optimized method was then used in time−course studies in pilot culture fields under controlled conditions of herbicide applications, flooding, and drainage, providing valuable information about the fate of the herbicide. These results and the introduction of this technique will allow delineation of management practices that permit rice farmers to achieve good levels of production with minimal environmental impact. Also, the environmental protection officers would take advantage of this approach.

MATERIALS AND METHODS

Materials. HPLC grade methyl alcohol (Baker, Phillipsburg, United States), phosphoric acid (BDH Chemicals Ltd., Poole, England), and HPLC grade clomazone (Riedel-de Haën, Seelze, Germany) were a used as primary standards for ELISA and HPLC. The herbicides quinclorac, atrazine, simazine, propanil, bispyribac, pirazosulfuronethyl, metsulfuron-methyl, imazethapyr, and glyphosate were from Cyperex (Uruguay). All other reagents were from Sigma (St. Louis, MO), unless specified otherwise.

Synthesis of Aminoclomazone (A-Clo) Conjugates. Clomazone (technical grade, Cibeles Co., Uruguay) was conjugated to keyhole limpet hemocyanin (KLH, Pierce, Rockford, IL) or electrophoresis grade bovine serum albumin (BSA) as described by Koppatschek et al. (*11*). Briefly, 0.3 mL of concentrated nitric acid and 1.2 mL of concentrated sulfuric acid were mixed and added dropwise to a stirred solution of 1.19 g of clomazone in 4 mL of sulfuric acid at −10 °C, over 30 min. The reaction was followed by thin-layer chromatography (TLC) employing ethyl-acetate:hexane (1:3) as the solvent. The nitration mixture was warmed to 0 °C and poured carefully into 24 mL of an ice/water mixture. Concentrated ammonium hydroxide was added to the mixture, resulting in the precipitation of the nitrated product. The precipitate was removed by filtration, washed with cold 1 M HNO_3, and air-dried. Incorporation of the nitro group to clomazone was confirmed by NMR. Then, 0.3 mg of platinum oxide catalyst was added to 0.27 g of nitro-clomazone in 78 mL of ethanol. The mixture was shaken in a 250 mL thick-walled bottle on a Parr hydrogenator under hydrogen at 45 psi. After 60 min, the pressure dropped to 2 psi. The resulting clear, yellow solution was filtered through filter paper to remove the catalyst, and the solvent was removed below 30 °C under reduced presure. The reduction of nitro-clomazone to A-Clo was evaluated by NMR. The conjugation of A-Clo to the carrier proteins was done on ice. Ten milligrams of A-Clo was added to 1.5 mL of 0.1 N HCl, followed by dropwise addition of 0.052 M $NaNO_2$ until a positive starch−iodine test was obtained. After 30 min of additional stirring, 0.5 mL of the reaction mixture was added to 0.02 g of carrier protein dissolved in 2.5 mL of 0.1 M borate buffer, pH 9.0. The mixture was stirred for 2 h, with regular addition of 0.1 N NaOH to maintain the pH close to 9.0. The bright orange reaction mixture was dialyzed for 24 h against phosphate-buffered saline (PBS), pH 7.5.

Synthesis of 2-{2-Chloro-4-[(4-hydroxyphenyl)diazenyl]benzyl}-4,4-dimethyl-1,2-oxazolidin-3-one (Phe-Clo) Conjugates. Ten milligrams of A-Clo was added to 1.5 mL of 0.1 N HCl, followed by dropwise addition of 0.052 M $NaNO_2$ until a positive starch−iodine test was obtained. After 30 min of additional stirring, 0.5 mL of the reaction mixture was combined with 2.5 mL of 0.039 mmol of phenol dissolved in 0.1 M borate buffer, pH 9.0. The mixture was stirred for 2 h, with regular addition of 0.1 N NaOH to maintain the pH close to 9.0, and then, 0.1 N HCl was added dropwise to pH 2.0, to induce precipitation of the product. The resulting adduct was filtered and purified by preparative TLC, and its structure was confirmed by NMR. The hydroxyl group of the phenol spacer was used to conjugate this hapten to BSA and ovalbumin (OVA) (*12*). Briefly, 5 mg of Phe-Clo was dissolved in 1 mL of DMSO, and 200 μL of this solution was activated with 8.8 mg of CDI (1,1′-carbonyl-diimidazole, Pierce) for 2 h in the dark. Then, 200 μL of the carrier protein (5 mg in 100 mM $NaHCO_3$, pH 8.5) was added slowly under continuous stirring and allowed to react overnight at 4 °C. Conjugation was evaluated by UV spectrometry (not shown).

Antibody Production. One hundred micrograms of KLH-A-Clo or OVA-Phe-Clo was dissolved in 250 μL of PBS and vigorously mixed with 250 μL of Freund's complete adjuvant (Pierce) to form a thick emulsion. This emulsion was then injected subcutaneously into several points on the back of New Zealand white rabbits. After 4 and 8 weeks, the animals were immunized intramuscularly with additional doses of 100 μg of hapten conjugates emulsified in Freund's incomplete adjuvant. Ten days after the final booster, the animals were bled, and after they were clotted and centrifuged, the sera were aliquoted and kept at −20 °C until used.

Checkerboard Titration. Polystyrene high-binding microtiter plates (Greiner bio one, Germany) were coated with 100 μL of serial dilutions of coating antigen in PBS, overnight at 4 °C, starting with 1 μg/mL in the first row, 0.5 μg/mL in the second, and so on. The plates were washed three times with PBS−0.05% Tween 20 (PBS-T) and blocked by incubation with PBS−0.1% Tween 20 for 1 h. Then, 100 μL of serial dilutions of rabbit sera in PBS-T was dispensed, starting with a 1/100 dilution in the first column, 1/200 in the second, and so on. After 1 h at room temperature, the plates were washed three times with PBS-T, and 100 μL of antirabbit IgG antibody conjugated to horseradish peroxidase (HRP) (Pierce) diluted 1/5000 in PBS-T. After 1 h of incubation at room temperature, the plates were washed five times with PBS-T, and the wells were loaded with 100 μL of peroxidase substrate (0.4 mL of 6 mg 3,3′,5,5′-tetramethylbenzidine in 1 mL of DMSO and 0.1 mL of 1% H_2O_2 in water in a total of 25 mL of 0.1 M citrate acetate buffer, pH 5.0) and incubated for 20 min at room temperature. The enzyme reaction was stopped by the addition of 50 μL of 2 N H_2SO_4, and the absorbance was read at 450 nm in a microtiter plate reader (FLUOstar OPTIMA, BMG Labtech, Germany)

Clomazone ELISA. On the basis of checkerboard titration, the appropriate dilutions of coating antigen and antibody were selected to obtain a maximum absorbance reading close to 1 absorbance unit (AU). The competitive ELISA was then set up essentially as described above. Briefly, 96-well microtiter plates were coated and washed as described, and then, 50 μL/well of clomazone standard or sample was combined with 50 μL of the appropriate dilution of the antibodies. After 1 h of incubation at room temperature, the plates were washed and developed as above. All dilutions were performed with PBS-T except when interference buffer (1 M Tris, 0.3 M NaCl, 0.3 M EDTA, and 1% BSA, pH 7.4) was used. The software employed for the analysis of data was Origin 7.5

HPLC Analysis. The equipment used consisted of a Waters HPLC (Milford, MA) model 1525 Binary HPLC Pump with a 20 μL sample loop, associated with a Waters 2487 Dual λ Absorbance Detector. The analytical column was a Phenomenex (Torrance, CA) 5 μm C18, of 250 mm × 4.60 mm. The samples were analyzed as described by Zanella et al. (*13*) with modifications. The analytical column was operated isocratically at room temperature, using methyl alcohol:Milli-Q water (65:35) acidified to pH 3.0 with phosphoric acid. The flow rate was set at 0.8 mL/min, and runs were monitored by UV detection at 220 and 254 nm. A standard curve with 0.1, 1.0, and 8.0 μg/mL of clomazone was built and used for quantization. Water samples were preconcentrated by adsorption on SPE columns (Strata C18-E from Phenomenex). Briefly, water samples were filtered with Whatman paper 1 and then with 0.4 μm cellulose membranes (Millipore, Bedford, MA), and the pH was adjusted to 3.0 by the addition of phosphoric acid. Then, the SPE column was activated by passing consecutively 3 mL of methanol, 3 mL of Milli-Q water, and 3 mL of Milli-Q water, pH 3, and 250 mL of water was applied to the column at 5 mL/min. After that, the SPE column was eluted with 1 mL of methanol, which was used for analysis.

Pilot Studies of Clomazone Dissipation. The persistence of the herbicide was studied in experimental rice cultures, implemented by the

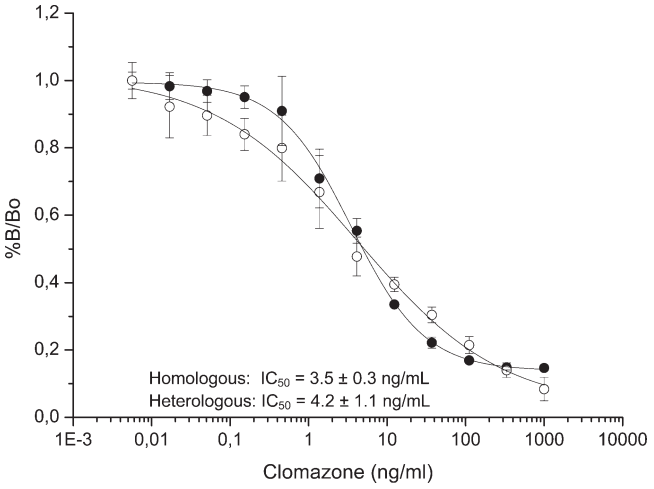


Figure 1. Clomazone competitive ELISAs using PBS as reaction buffer. The homologous (black) and heterologous (white) assays were set up using A-Clo-BSA and Phe-Clo-BSA, respectively. The concentration causing 50% inhibition (IC_{50}) was 3.5 ± 0.3 ng/mL for the homologous assay and 4.2 ± 1.1 ng/mL for the heterologous assay, with detection limits of 0.9 and 1.1 ng/mL, respectively.

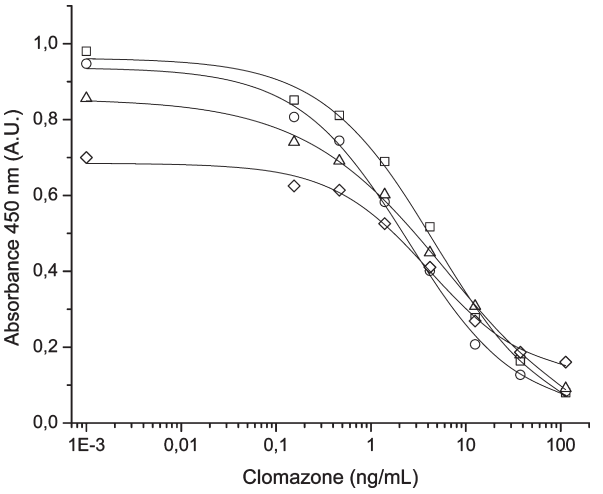


Figure 2. Effect of interference buffer on assay performance. Four conditions were tested using 0 (squares), 5 (circles), 10 (triangles), or 20% (diamonds) interference buffer in the competition step of the ELISA.

National Institute of Agricultural Research (INIA) during the harvest 2008/2009 in Treinta y Tres, Uruguay. The pilot experiments were designed to evaluate the effect of two different irrigation treatments on the fate of the herbicide. During the study, climatic variables were monitored in the Meteorological Station of the experimental unit. Two adjacent plots of 112 m^2 were used. Clomazone, 384 g/ha as an emulsifiable commercial formulation, 120 L/ha of broth, was applied to both plots at the same moment 13 days postemergence of the culture. After 2 days, plot #1 was flooded with a water layer of 10 cm (early flooding), which was maintained during the flooding cycle. Plot #2 was flooded under the same conditions but 16 days after application, which is the standard procedure used by local rice farmers. Samples were regularly collected from the water layer. The samples were maintained at 4 °C and analyzed within the following week.

RESULTS AND DISCUSSION

Antibody Production and Checkerboard Titration. The IgG titer of New Zealand rabbits immunized with A-Clo-KLH was determined by indirect ELISA, on plates coated with A-Clo-BSA, Phe-Clo-BSA, or BSA as a negative control. Very high titers were obtained against the A-Clo conjugate (but not to Phe-Clo), and no reactivity with BSA was observed. The animal showing the highest response to the hapten was chosen. The serum titer (midpoint of the titration curve) was 500-fold higher for A-Clo than Phe-Clo, showing that the bulky spacer somehow interfered with the binding of the clomazone moiety to the antigen binding site of the antibody.

Standard Curve of the Clomazone ELISA. The amount of coating antigen and proper dilution of the antibody were established by checkerboard titration as described above. The serum dilution producing an ELISA reading close to 1 AU with the smallest amount of coating antigen was chosen. This was obtained with 8.0 ng/mL of coating antigen and a serum dilution of 1/40000 for A-Clo-BSA and 1.0 μg/mL of coating antigen and a serum dilution of 1/8000 for Phe-Clo-BSA. The standard curves of the homologous assay (immunizing and coating hapten are the same) and heterologous assay (immunizing and coating hapten differ) are shown in **Figure 1**. The homologous ELISA had a midpoint (inhibitory concentration causing 50% inhibition, IC_{50}) of 3.5 ± 0.3 ng/mL with a limit of detection (LOD = IC_{20}) of 1.1 ng/mL. Similarly, the heterologous ELISA presented an IC_{50} = 4.2 ± 1.1 ng/mL and LOD = 0.9 ng/mL. This result was somewhat unexpected because, in general, heterologous assays set up with polyclonal antibodies tend to produce assays with improved sensitivity (*14*, *15*).

Both ELISAs were then used to validate the method with real water samples. Five samples were collected from the source river or rice fields where clomazone had not been used and were spiked with various concentrations of the herbicide. The results (not shown) were unsatisfactory with unacceptable recoveries for both methods. On the basis of our previous experience, the inclusion of a bulk protein that may interact with humic acids, chelating agents that sequester metal ions, and an increase ionic strength may help to decrease the matrix effect. To test this hypothesis, different proportions of "interference buffer", 1 M Tris, 0.3 M NaCl, 0.3 M EDTA, and 1% BSA, pH 7.4, were included during the competition step of the assay. **Figure 2** shows the standard curves of the homologous method performed under these conditions, the inclusion of interference buffer causes a moderate decrease of the maximum signal, but the parameters of the curve are basically not modified. Initial experiments demonstrated that the best recoveries were obtained when 10% of interference buffer was used; therefore, this buffer composition was selected to validate the assays. **Figure 3** displays the results obtained with the homologous assay. In the 2−20 ng/mL range, there was a good correlation ($y = 0.94x + 0.23$, $R^2 = 0.85$) between the amount of clomazone used for spiking and its measured concentration in four different water samples. Similar recoveries were obtained with the heterologous method; however, the latter appeared to be less robust and was not further studied.

Assay Specificity. The specificity of the assay was tested using a panel of herbicides that are representative of the compounds currently used in rice agriculture in Uruguay. The cross-reactivity of the assay for each compound was calculated as % cross-reactivity = 100[IC_{50} (clomazone)/IC_{50} (cross-reacting compound)] (**Table 1**). Except for the clomazone-related hapten synthesized in this study, none of the other herbicides exhibited significant cross-reactivity at the highest concentration tested.

Intermethod Comparison. The assay was also validated by comparing ELISA and HPLC clomazone measurements in representative water samples. Clomazone was assayed in 15 water samples taken from various local rice fields at different time points along the harvest. Four samples were below the LOD of both methods, and for the rest, there was a very good correlation between both techniques ($y = 0.98x - 0.15$, $R^2 = 0.98$) (**Figure 4**). The fact that both methods produced equivalent results in the 2−18 ng/mL range is of great relevance for this study, particularly when the simplicity of the ELISA technique is considered. Indeed,

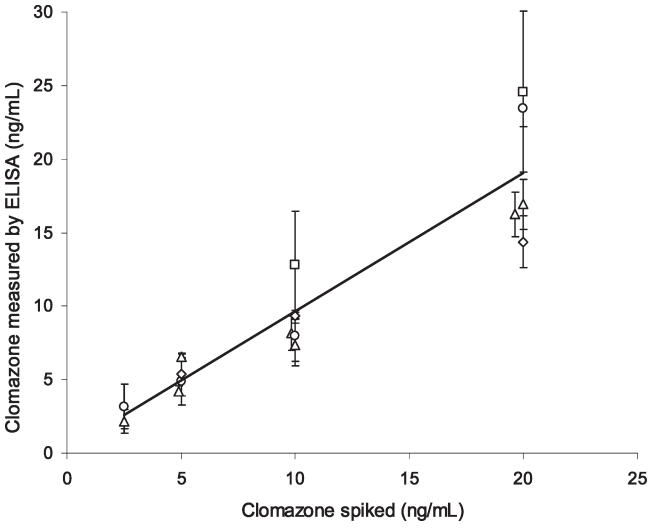
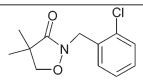
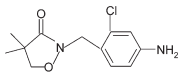
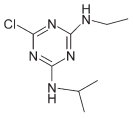
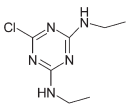
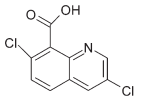
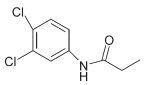
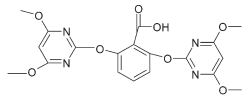
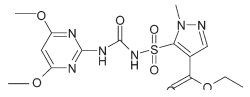
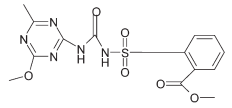
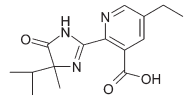
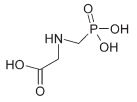


Figure 3. Recovery of spiked water samples. Four water samples collected from the source river (circles), a rice field where clomazone had not been used (diamonds), and two flooding channels (triangles and squares) were spiked with various concentrations of clomazone and then analyzed by ELISA.

HPLC analysis requires tedious filtering steps and passage of large water volumes through preconcentration columns, while on the other hand a large number of a small-volume (1 mL) water samples can be readily analyzed in parallel by ELISA after a 2 min centrifugation step.

Dissipation of Clomazone in Water Samples from Rice Culture. The dissipation of the herbicide was analyzed in a water management study implemented at El Paso de la Laguna Experimental Unit (33° 16′S 54° 10′W) of the National Institute of Agricultural Research during the 2008−2009 harvest in Treinta y Tres, Uruguay. An important aim of this project is to define the best conditions of flooding and drainage that optimize productivity, while minimizing the release of agrochemicals into the environment. The assay developed in this work was used in an initial model experiment in which two culture plots were flooded at different times after application of the herbicide as described. As expected, early irrigation of the plot was accompanied by high concentrations of clomazone in the water layer (**Figure 5**). After an initial concentration of 77 ng/mL found the day of flooding, there was a temporary reduction, in the first 3 days, to a minimum value of about 45 ng/mL, at which time the clomazone concentrations increased rapidly to reach a maximum of 129 ng/mL at day 6 after flooding. If we consider an irrigation water layer thickness of 10 cm, this concentration corresponds to approximately one-third of the total amount applied to the plot. The amount of clomazone decreased afterward, until day 41, when the concentration of the herbicide in the water layer was below the LOD. Late irrigation was associated with lower concentrations of clomazone, peaking at day 9 after flooding (26 ng/mL) and waning until day 30 when the compound became undetectable. There was a remarkable difference between the two conditions of irrigation. Apparently, late irrigation, which is the procedure commonly used by local farmers, favors stronger herbicide adsorption onto the soil, its microbial or photoinduced degradation, or its volatilization (*16*), which should be further studied. The high levels of clomazone attained with early irrigation may be a matter of concern, and care should be taken to avoid that climatic conditions, such as heavy rain, may cause uncontrolled release of the herbicide during peak days. On the basis of studies performed by the Californian Department of Pesticide Regulation (*17*), it has been recommended that growers withhold treated water in their farms for a minimum of 21−28 days, to minimize environmental impacts associated with herbicides. Under the conditions investigated in our study, the residues of herbicide reached environmentally safe levels 40 and 30 days after flooding, for the early and late irrigation treatments, respectively, which reveals the importance of local monitoring of these parameters.

Table 1. Cross-Reactivity of Agrochemicals Currently Used in Rice Production in Uruguay

Herbicide name	Herbicide structure	% Cross-reactivity
Clomazone		100
Aminoclomazone		74
Atrazine		< 0.8
Simazine		< 0.8
Quinclorac		< 0.8
Propanil		< 0.8
Bispyribac		< 0.8
Pirazosulfuronethyl		< 0.8
Metsulfuron methyl		< 0.8
Imazethapyr		< 0.8
Glyphosate		< 0.8

These pilot experiments showed the potential of the method, producing the first set of data about the fate of the herbicide in the production system used in Uruguay. On the basis of its robustness and simplicity, the method could become a useful tool to facilitate further optimization of management practices that will allow farmers to achieve good production standards, while preserving the environment. The method could be particularly suitable for monitoring water quality in rice-growing regions of developing countries that currently are not able to do such water testing due to cost or lack of capability to perform instrumental analysis. In previous studies, we have demonstrated the potential of other ELISA formats to allow broader uses of the method than the type of assays used in this study. For example (*18*), dipstick formats

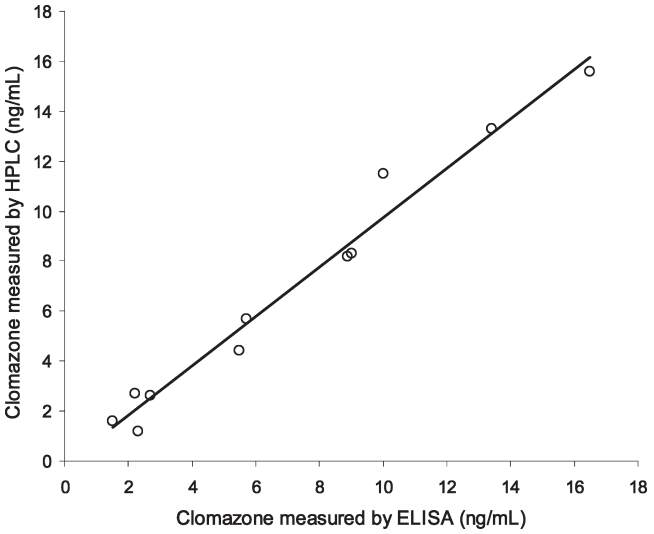


Figure 4. Comparison of ELISA and HPLC determination of clomazone in field samples collected at different time points along the harvest. Samples were analyzed in triplicate (ELISA) and duplicate (HPLC).

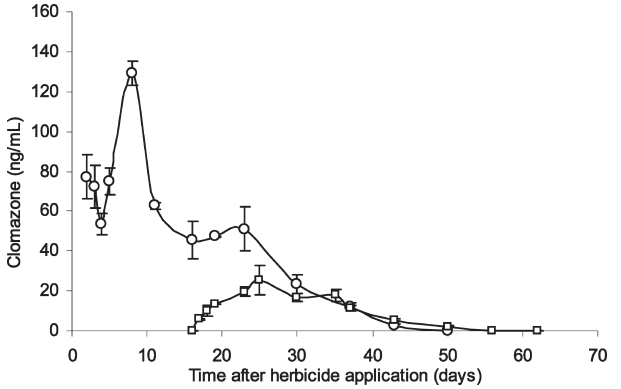


Figure 5. Concentration of clomazone in pond water of two rice plots cultivated under different conditions of irrigation. Plot 1 (circles) and plot 2 (squares) were flooded 2 and 16 days after application of the herbicide, respectively, and maintained with a 10 cm water layer throughout the study.

might be developed that would allow the farmer who is not trained as an analytical chemist to do semiquantitative assays at the point of use of the herbicide. The ability to actually monitor the clomazone content of the irrigation water under local conditions prior to release of the water to the environment can ensure the use of "best practices" under a wide range of field conditions. We are currently exploring the adaptation of the clomazone immunoassay into Phage anti-immunocomplex assay (PHAIA), which can increase the sensitivity by 5−20-fold, and it is easily adapted into rapid formats (*18*, *19*).

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LITERATURE CITED

(1) Lattimore, M.-A. Pastures in temperate rice rotations of south-eastern Australia. *Aust. J. Exp. Agric.* **1994**, *34*, 959–965.

(2) Saldain, N.; Deambrosi, E. Selectividad del Command en preemergencia en El Paso 144. In *Arroz Resultados Experimentales 2008−2009—Instituto Nacional de Investigación Agropecuaria*; Alvarez, O., Ed.; INIA: Treinta y Tres, Uruguay, 2009; pp 7−12.

(3) Li, L. F.; Li, G. X.; Yang, R. B.; Guo, Z. Y.; Liao, X. Y. Clomazone dissipation, adsorption and translocation in four paddy topsoils. *J. Environ. Sci.* (*China*) **2004**, *16*, 678–682.

(4) Quayle, W. C.; Oliver, D. P.; Zrna, S. Field dissipation and environmental hazard assessment of clomazone, molinate, and thiobencarb in Australian rice culture. *J. Agric. Food Chem.* **2006**, *54*, 7213–7220.

(5) Crestani, M.; Menezes, C.; Glusczak, L.; dos Santos Miron, D.; Spanevello, R.; Silveira, A.; Goncalves, F. F.; Zanella, R.; Loro, V. L. Effect of clomazone herbicide on biochemical and histological aspects of silver catfish (*Rhamdia quelen*) and recovery pattern. *Chemosphere* **2007**, *67*, 2305–2311.

(6) EEC Drinking Water Directive, European Economic Community, Brussels. *80/778/EEC, EEC N° L229/11−29*, 1980.

(7) Slobodnik, J.; Louter, A.; Vreuls, J.; Liska, I.; Brinkman, U. A. Monitoring of organic micropollutants in suface water by automated on-line trace-enrichment liquid and gas chromatographic system with ultraviolet diode-array and mass spectrometric detection. *J. Chromatogr. A* **1997**, *768*, 239–258.

(8) Sherma, J. Pesticide residue analysis (1999−2000): A review. *J. AOAC Int.* **2001**, *84*, 1303–1312.

(9) Brena, B. M.; Arellano, L.; Rufo, C.; Last, M. S.; Montano, J.; Egana Cerni, E.; Gonzalez-Sapienza, G.; Last, J. A. ELISA as an affordable methodology for monitoring groundwater contamination by pesticides in low-income countries. *Environ. Sci. Technol.* **2005**, *39*, 3896–3903.

(10) Lucas, A. D.; Gee, S. J.; Hammock, B. D.; Seiber, J. N. Integration of immunochemical methods with other analytical techniques for pesticide residue determination. *J. AOAC Int.* **1995**, *78*, 585–591.

(11) Koppatscheck, F.; Liebl, R.; Alan, L.; Kriz, A.; Melhado, L. Development of an enzyme-linked immunosorbent assay for the detection of the herbicide clomazone. *J. Agric. Food Chem.* **1990**, *38*, 1519–1522.

(12) Tudos, A. J.; Lucas-van den Bos, E. R.; Stigter, E. C. A. Rapid surface plasmon resonance-based inhibition assay of deoxynivalenol. *J. Agric. Food Chem.* **2003**, *51*, 5843–5848.

(13) Zanella, R.; Primel, E. G.; Goncalves, F. F.; Martins, A. F. Development and validation of a high-performance liquid chromatographic method for the determination of clomazone residues in surface water. *J. Chromatogr. A* **2000**, *904*, 257–262.

(14) Ahn, K. C.; Watanabe, T.; Gee, S. J.; Hammock, B. D. Hapten and antibody production for a sensitive immunoassay determining a human urinary metabolite of the pyrethroid insecticide permethrin. *J. Agric. Food Chem.* **2004**, *52*, 4583–4594.

(15) Shan, G.; Huang, H.; Stoutamire, D. W.; Gee, S. J.; Leng, G.; Hammock, B. D. A sensitive class specific immunoassay for the detection of pyrethroid metabolites in human urine. *Chem. Res. Toxicol.* **2004**, *17*, 218–225.

(16) David Gara, P. M.; Bosio, G. N.; Arce, V. B.; Poulsen, L.; Ogilby, P. R.; Giudici, R.; Gonzalez, M. C.; Martire, D. O. Photoinduced degradation of the herbicide clomazone model reactions for natural and technical systems. *Photochem. Photobiol.* **2009**, *85*, 686–692.

(17) California Department of Pesticide Regulation, C. P. R. *California Department of Pesticide Regulation, Clomazone Public Report 200301*, 2003.

(18) Gonzalez-Techera, A.; Vanrell, L.; Last, J. A.; Hammock, B. D.; Gonzalez-Sapienza, G. Phage anti-immune complex assay: General strategy for noncompetitive immunodetection of small molecules. *Anal. Chem.* **2007**, *79*, 7799–7806.

(19) Gonzalez-Techera, A.; Kim, H. J.; Gee, S. J.; Last, J. A.; Hammock, B. D.; Gonzalez-Sapienza, G. Polyclonal antibody-based noncompetitive immunoassay for small analytes developed with short peptide loops isolated from phage libraries. *Anal. Chem.* **2007**, *79*, 9191–9196.

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