

# Development of an immunological technique for the detection of clomazone and its use in the monitoring of rice culture

Mariana Carlomagno<sup>1</sup>, Cecilia Mathó<sup>1</sup>, Guillermina Cantou<sup>2</sup>, David González<sup>1</sup>, Alvaro Roel<sup>2</sup> y Gualberto González-Sapienza<sup>1</sup>  
<sup>1</sup> Cátedras de Inmunología y Química Orgánica, Facultad de Química, Universidad de la República, Montevideo, Uruguay. [maricarl@cin.edu.uy](mailto:maricarl@cin.edu.uy)  
<sup>2</sup> Instituto Nacional de Investigación Agropecuaria (INIA), Treinta y Tres, Uruguay.

## INTRODUCTION

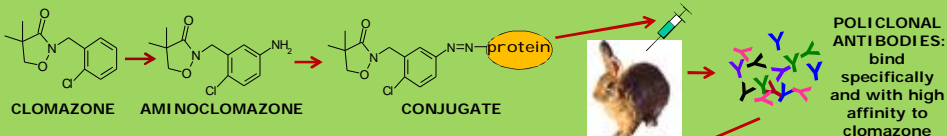
Rice culture in Uruguay is based in general in a system of production in rotation with pastures, what allows it to be considered of low intensity and small environmental impact. To quantify the environmental compatibility of the system it is necessary to determine the residue levels of agrochemicals. In Uruguay, the herbicide clomazone is one of the most employed, being applied to 78% of the rice area. It is selective to rice cultures, of pre and post emergence, for the control of graminneas (*Echinochloa crusgalli*, *Echinochloa crusgavonis*, *Digitaria sanguinalis* and *Echinochloa colona*). The maximum limit permitted for the herbicide in surface waters is 3 ppb (ng.mL<sup>-1</sup>). Generally, clomazone is detected by High Performance Liquid Chromatography (HPLC) or gas chromatography, methods that require a demanding clean up of the sample, and expensive equipment.

Immunoassays (ELISA) are a complementary analytical tool of low cost, and allow the analysis of many samples in a short time. Immunoassays employ antibodies which are molecules that recognize specifically and with high affinity virtually any molecular structure. The introduction of a cost efficient assay for the detection of clomazone in the rice production field will allow, through monitoring, to detect and correct possible management practices having a negative impact on the environment.

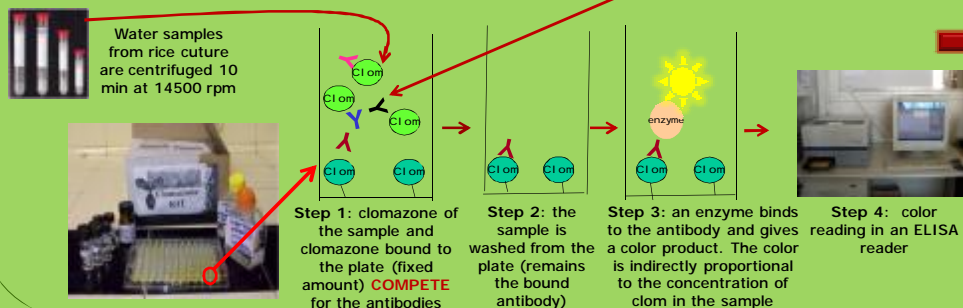
## METHODOLOGY

### 1. DEVELOPMENT OF A COMPETITION ELISA

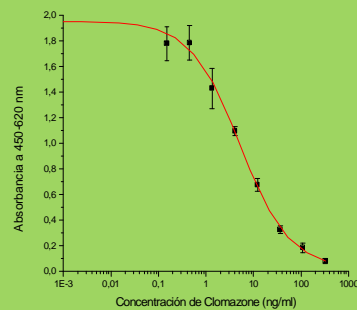
#### 1. Synthesis of clomazone hapten, conjugation and preparation of antibodies



#### 2. Optimization: Serum dilution: 1/40.000, Coating: 8 ng/mL



## RESULTS



Limit of detection (LOD) = 1.0 ± 0.4 ng/mL

IC50: 5,0 ± 1,4 ng/mL

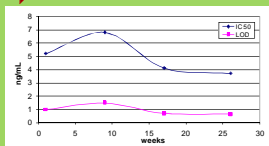
**ELISA: 60 samples (triplicates) analyzed in 9 hs !!!**

### 2. VALIDATION OF THE ELISA

#### 1. Fortification assays

Clomazone was detected in water samples of rice culture added at ≥3,0 ng/mL, recoveries between 70-120%

#### 2. Stability of the kit in time



#### 3. Cross reactivity

No reactivity (IC50 > 1000) with: atrazine, quinclorac, simazine, propanil, bispiribac, pirazosulfuronetil, metsulfuron methyl, imazethapir, amonic salt of glyphosate, isopropylamine glyphosate salt.

#### 3. ELISA vs HPLC



Water samples are filtered with:  
 • whatman Nº1 and  
 • filter paper 0.4 µm



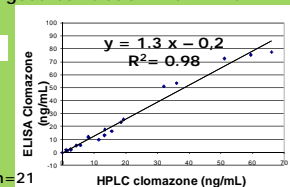
Samples are passed through C18 columns that retain clomazone



Extracts are analyzed by HPLC. Solvent: 65% methanol

ELISA showed a good correlation with HPLC

HPLC LOD: 0.5 ng/mL



**20 mL methanol per sample**

**HPLC: 6 samples (duplicates) analyzed in 11 hs !!!**

### 3. DISSIPATION OF HERBICIDES IN RICE CULTURE

Determine the concentration of clomazone and quinclorac in water of rice culture and evaluate its interaction with culture water management in two flooding treatments.



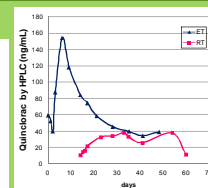
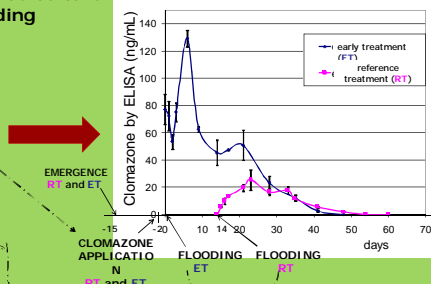
Experimental culture: implemented by INIA in Treinta y Tres, Uruguay, harvest 2008/2009. Plots of 112 m<sup>2</sup>, during the culture rice is maintained flooded with 10 cm layer of water.

Clomazone and quinclorac application: 384 g.ha<sup>-1</sup> from an emulsifiable commercial formulation, in 120 L.ha<sup>-1</sup> of broth, in RT and ET.

#### Two flooding treatments:

1. Reference treatment (RT): flooding the culture 30 days after emergence (commonly used in production).
2. Early treatment (ET): flooding the culture 15 days after the emergence.

Water samples were collected of the water layer of the plots, clomazone was detected by ELISA and quinclorac by HPLC.



Quinclorac dissipation is very similar to that of clomazone, with the exception that quinclorac is more persistent in time

For clomazone in RT and ET: necessary to wait 35 and 41 days after flooding, respectively, to reach levels lower than the accepted.

During the culture: lower level of herbicides in RT compared to ET, shows that herbicides were dissipated in RT when they were on the soil without flooding (days 0 to 14).

Before flooding RT were done baths in the culture, in which water went out of the culture with 70 ng/mL clomazone and 80 ng/mL quinclorac!!

## CONCLUSIONS AND PERSPECTIVES

The ELISA developed for clomazone had a limit of detection of 1,0 ± 0.4 ng/mL and was validated against the reference methodology (HPLC). The technique is a fast, low cost and not harmful to the environment tool for the detection of agrochemicals.

The results obtained of the dissipation of clomazone and quinclorac in rice culture water proved that, to preserve the quality of the hydric resources, it is important to adopt management practices that avoid or minimize the movement of water out of the culture in the first days after flooding (specially at ET) and in the baths. This kind of study, provides information about how the actual management practices interact with the levels of dissipation of agrochemicals, and constitute the initial step to delineate good management practices that allow to achieve good levels of production, preserving the environment.