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# Occurrence and Distribution Study of Residues from Pesticides Applied under Controlled Conditions in the Field during Rice Processing

Lucía Pareja,[†,§,‡] Marcos Colazzo,[†] Andrés Pérez-Parada,[†,§] Natalia Besil,[†] Horacio Heinzen,[†,‡] Bernardo Böcking,[⊗] Verónica Cesio,[†] and Amadeo R. Fernández-Alba*[,§]

[†]Cátedra de Farmacognosia y Productos Naturales, Facultad de Química, Universidad de la República, General Flores 2124, Montevideo, Uruguay

[§]Pesticide Residue Research Group, Department of Hydrogeology and Analytical Chemistry, University of Almeria, 04120 La Cañada de San Urbano, Almeria, Spain

[‡]Departamento de Química del Litoral, Polo Agroalimentario y Agroindustrial, Centro Universitario de Paysandú, Universidad de la República, Ruta 3, Km 363, Paysandú, Uruguay

[⊗]Donistar S.C., Col. Rubio, Salto, Uruguay

Supporting Information

**ABSTRACT:** The results of an experiment to study the occurrence and distribution of pesticide residues during rice cropping and processing are reported. Four herbicides, nine fungicides, and two insecticides (azoxystrobin, byspiribac-sodium, carbendazim, clomazone, difenoconazole, epoxiconazole, isoprothiolane, kresoxim-methyl, propanil, quinclorac, tebuconazole, thiamethoxam, tricyclazole, trifloxystrobin, λ-cyhalotrin) were applied to an isolated rice-crop plot under controlled conditions, during the 2009−2010 cropping season in Uruguay. Paddy rice was harvested and industrially processed to brown rice, white rice, and rice bran, which were analyzed for pesticide residues using the original QuEChERS methodology and its citrate variation by LC-MS/MS and GC-MS. The distribution of pesticide residues was uneven among the different matrices. Ten different pesticide residues were found in paddy rice, seven in brown rice, and eight in rice bran. The highest concentrations were detected in paddy rice. These results provide information regarding the fate of pesticides in the rice food chain and its safety for consumers.

**KEYWORDS:** *occurrence, distribution, rice processing, pesticide residues, LC-MS/MS*

## INTRODUCTION

Rice is one of the most consumed foods in the world, and its consumption has increased in recent decades, with a consequent rise in the use of pesticides, such as pre- and postemergence herbicides, insecticides, and fungicides, during various stages of the cultivation to improve its production yield.[1,2] Uruguay has a peculiar rice cultivation system that involves yearly rotation between rice cropping and prairies for cattle breeding. Additionally, Uruguay is the sixth largest exporter of rice in the world and, to maintain this privileged position, Good Agricultural Practices have been approved recently by Uruguayan rice growers, by which pesticide use is regulated.[3,4] The main pesticides employed are herbicides and fungicides, whereas few insecticides are used in rice crops. In general, their residues can persist until the harvest stage, resulting in the contamination of the rice grain. This fact has been already confirmed by the presence of pesticide residues in the final product.[5,6] Nevertheless, there is scarce information on the distribution of pesticide residues during crop growth and development as well as their occurrence after the raw material processing. The point has gained great interest worldwide, as it is of paramount importance from an environmental, nutritional, and toxicological point of view. The portion of pesticides that remains in the environment dealt with the sustainability of the agro-ecosystem. Moreover, the amount of residue incorporated in production could have noxious effects on human or animal health. The maximum residue limits (MRLs) are trading standards. However, there is no universal agreement among the different regulatory organizations on which combinations of commodity/pesticide and maximum concentration are allowed. The *Codex Alimentarius* set the MRLs in different rice commodities, but it does not include all of the new pesticides used in the technological package in the different countries. On the other hand, the European Union (EU) also establishes MRLs, for a higher number of pesticides on "rice", because it sets a default value for those pesticides that are not included in Annex 1. Moreover, the United States regulates some other pesticides and establishes MRLs different from those of the *Codex* and EU.[7−9]

The processing of food commodities generally implies the transformation of the raw material aiming to give the product an added value that has greater shelf life and is closer to being

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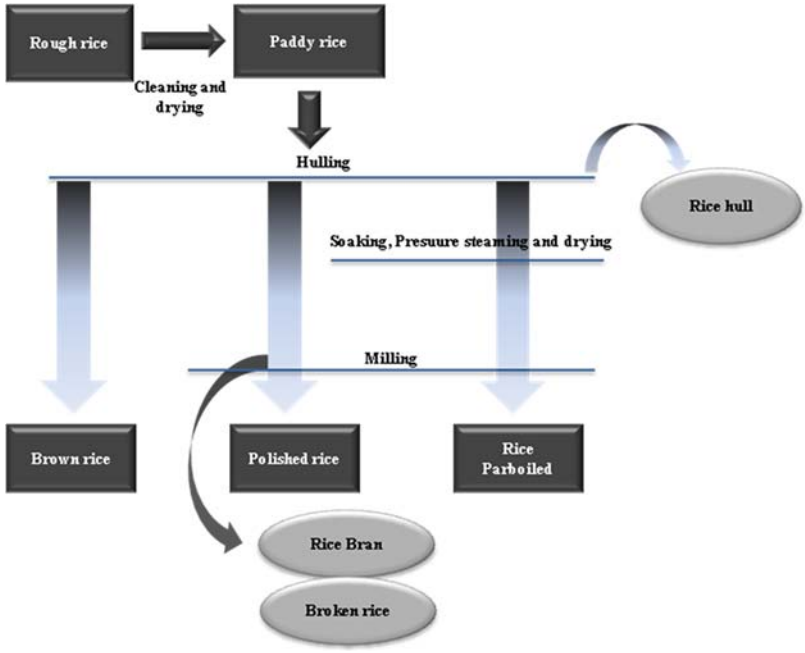


**Figure 1.** Scheme of rice processing.

table ready.[10] Unit operations that are normally employed in processing food crops generally reduce or remove residues of pesticides that are present in them.[11] In the case of rice crops, several steps are necessary to produce the final product. After harvest, the rough rice is cleaned and dried to reduce the moisture to around 14% to obtain paddy rice. Afterward, paddy rice is subjected to different processes depending on the desired product, white or brown rice. Brown rice is obtained by a primary milling operation, which includes a dehulling process of the paddy rice, whereas polished rice is obtained after dehulling and removal of bran layers (polishing).[12,13] As shown in Figure 1, three main types of rice are produced and marketed, paddy, brown, and white rice; however, during rice processing and depending upon the rice mill, other byproducts are obtained, the hull, bran rice, and broken rice.[13,14] Some of these commodities are used as common ingredients in horticultural, livestock, industrial, household, and food products.[12]

In most food-processing chains, pesticide residues suffer a large reduction during the industrial process, but, in the case of cereals, their diminution is lower.[6,14] Particularly for rice, there are several reports on the changes in the concentration of pesticides after different processes, such as milling, cooking, parboiling, and washing.[15,16]

However, up to now, there are few studies on the fate of pesticides involving several stages of rice processing. Therefore, little is known on which proportion the pesticide originally applied in the field could be found in the whole paddy rice grain and how the agrochemicals distribute between the finally obtained polished rice, rice bran, and brown rice.

The pesticide distribution during rice processing may depend on the chemical composition of each commodity obtained after the industrial process and their physicochemical properties. In 1993, Cogburn et al.[15] studied the distribution of malathion and chlorpyrifos on rough rice, hulls, brown rice, milled rice, and cooked rice and concluded that parboiling reduced residues on rough rice and hulls but tended to increase residues in the other obtained fractions. The information is even scarcer when considering the real case on the fate of fungicides, herbicides, and insecticides that are applied during the cropping season, their distribution during processing, and residue occurrence in the final products.

Recently, Brazilian workers studied the distribution of bispyribac sodium, clomazone, tebuconazole, and carbofuran in white rice, rice bran, husked rice, parboiled rice, parboiled rice bran, and husked parboiled rice from paddy rice, which was planted under controlled conditions. They describe the occurrence of all of the pesticide residues in the different rice commodities, some of them at relatively high concentration.[16]

In the present study we present our results on the occurrence and distribution in several commodities obtained after the milling process of paddy rice, namely, brown rice, rice bran, and white rice. Fifteen pesticides were studied including 4 herbicides, 9 fungicides, and 2 insecticides that were applied under controlled conditions to a rice-crop plot in Uruguay.

## MATERIALS AND METHODS

**Reagents.** HPLC-grade acetonitrile was purchased from J. T. Baker (Deventer, The Netherlands). A Milli-Q Plus ultrapure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the ultrapure water used during the analyses. Certified anhydrous $MgSO_4$ and ACS grade anhydrous NaAc were obtained from Panreac (Barcelona, Spain) and Riedel-de-Haën (Selze, Germany), respectively. The $MgSO_4$ was baked for 5 h at 500 °C in a muffle furnace to remove phthalates and residual water. NaCl and sodium citrate dehydrate were from J. T. Baker (Deventer, The Netherlands), whereas sodium citrate dibasic sesquihydrate was supplied by Sigma-Aldrich (St. Louis, MO, USA). Glacial acetic acid (HAc) was obtained from Merck (Darmstadt, Germany) and formic acid (98% purity) from Fluka (Steinheim, Germany). Solutions were prepared as needed. SPE sorbents, primary secondary amine (PSA) sorbent and C-18, 40 $\mu$m particle size, were supplied by Supelco (Bellefonte, PA, USA) and Varian (Palo alto, CA, USA) respectively.

Pesticide reference standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and Riedel-de-Haën (Selze, Germany) and were stored at −30 °C. Stock solutions of 1000−2000 mg/L of the individual standards were prepared in various solvents, four mix solutions of the pesticides were prepared from the stock solutions, and


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the working standard pesticide solutions were prepared daily by appropriate dilution of the four mix solutions with mobile phase and stored at −18 °C until use. A triphenyl phosphate solution (TPP) in MeOH was used as the surrogate standard in all of the LC experiments and as internal standard in the GC analyses.

Commercial products of the pesticides were purchased from a local supplier in Salto, Uruguay. The commercial products of the herbicides were Propanil 480 (propanil), Byspiriné (bispyribac sodium), Exocet, and Cibecol from Cibeles. The fungicides were Amistar (azoxystrobin) from Syngenta, Ipetec 40CE (isoprothiolane), Agrizim 500 (carbendazim), Convect 250 EC (difenoconazole), and Punch 75 WG (tricyclazole) from Agritec, Nativo 300SC (trifloxystrobin) from Bayer, Allegro (kresoxim-methyl + epoxiconazole) from BASF, and Bucaner 430F (tebuconazole) from Cibeles. Engeo 247 (thiamethoxam + λ-cyhalothrin) from Syngenta was applied as insecticide. As explained in the Introduction, the applied doses were 2 times higher than those recommended for rice.

**Instrumental and Chromatographic Conditions.** Liquid chromatography−electrospray ionization−tandem mass spectrometry, in positive and negative ion modes, was used for the separation and quantification of the analytes. For the LC analyses, an Agilent 1200 HPLC system (Agilent Technologies, Wilmington, DE, USA) with a binary pump was used. The analytical column employed was a reversed-phase C8 of 150 mm × 4.6 mm and 5 μm particle size (Agilent Zorbax Eclipse XDB). The mobile phases, A and B, were acetonitrile and high-purity water with 0.1% formic acid, respectively. The gradient program for the positive mode started with 20% B, constant for 3 min, followed by a linear gradient to 100% B in 30 min, and then constant for 3 min. After this 33 min run time, 12 min of post-time followed using the initial 20% B. For the negative mode the gradient program started with 50% B constant for 3 min, followed by a linear gradient to 100% B in 6 min, then constant for 3 min. After this 12 min run time, 5 min of post-time used the initial 50% B. The flow rate was constant, 0.6 mL/min during the whole process for both methods, and 10 μL of sample was injected in every case.

For the mass spectrometric analysis, an Agilent 6410 TripleQuad MS/MS system was used. The ESI source was operated in positive and negative ionization modes, and its parameters were as follows: gas temperature, 300 °C; gas flow, 9 L/min; nebulizer gas, 40 psi; and capillary voltage, ± 4000 V. Nitrogen served as the nebulizer and collision gas. For analysis in the positive mode, two segments with *a* ± 1 min overlapping range around the borders were constructed. The start times of the first and second segments in the positive mode were 0 and 18.2 min, respectively, whereas in the negative mode only one segment was used. Optimization of the compounds was performed by flow injection analysis (FIA), injecting individual standard solutions directly into the source. Table S1 in the Supporting Information shows the values of the instrumental settings optimized for each compound: fragmentation voltage (V) for precursor ions and collision energy (CE) for product ions. For the identification of the studied compounds two SRM transitions and a correct ratio between the abundances of the two optimized SRM transitions (SRM2/SRM1) were used, along with retention time matching.

Agilent Mass Hunter Data Acquisition, Qualitative Analysis and Quantitative Analysis software, was used for method development and data acquisition.

Total ion chromatograms in the full-scan mode were obtained by using a high-performance liquid chromatography (HPLC) system (consisting of a vacuum degasser, an autosampler, and a binary pump) (Agilent series 1100, Agilent Technologies, Santa Clara, CA, USA) equipped with a reversed-phase XDB-C18 analytical column of 4.6 mm × 50 mm and 1.8 μm particle size (Agilent Technologies). An amount of 20 μL of the sample extract was injected in each run. Mobile phases A and B were water/acetonitrile (95:5, v/v) with 0.1% formic acid and MeCN/water (95:5, v/v) with 0.1% formic acid. The chromatographic method held the initial mobile phase composition (10% B) constant for 1 min, followed by a linear gradient to 100% B up to 12 min and kept for 5 min at 100% B. The flow rate used was 0.6 mL/min. The HPLC system was connected to a time-of-flight mass spectrometer Agilent MSDTOF (Agilent Technologies) equipped with an electrospray interface operating in the positive mode, using the following operation parameters: capillary voltage, 4000 V; nebulizer pressure, 40 psi; drying gas flow rate, 9 L/min; gas temperature, 325 °C; skimmer voltage, 60 V; octapole dc, 37.5 V; octapole rf, 210 V; fragmentor voltage (in-source CID fragmentation). LC-MS accurate mass spectra were recorded across the range of $m/z$ 50−1000.

GC-MS analyses were performed using an HP 6890 GC coupled with an HP 5973 mass spectrometer supported by reference libraries, equipped with an HP-5 (5% diphenyl, 95% dimethylsiloxane) bonded fused-silica capillary column (25 m × 0.25 mm i.d. × 0.25 μm film thickness). Electron impact (EI) mass spectra were obtained at 70 eV and programmed in selected ion monitoring (SIM) mode as indicated in Table S2 of the Supporting Information. The working parameters were as follows: injector temperature, 280 °C; interface temperature, 250 °C; carrier gas, He at 38 cm/s (1 mL/min); oven conditions, from 120 °C initial (hold for 5 min), increased to 285 °C at a rate of 5 °C/min (3 min hold). One microliter of the sample was injected in the splitless mode. The identification of the compounds was confirmed by injection of pure standards and comparison of their retention index and relevant MS spectra. Quantitation was carried out using TPP internal standard at a level of 1 μg/mL by calculating the pesticide response factor.

**Field Experiment.** *Preparation of the "Treated" Material.* The rice used in this study was cropped and harvested in northwestern Uruguay (31° 38′ S, 57° 96′ W) in a 10 × 25 m (250 $m^2$) field, after the application of a total of 15 agrochemicals commonly used in rice. The pesticide doses and the application times are shown in Table 1.

**Table 1. Agrochemical Products and Treatment Dose**

| active substance | no. application; month | application rate (L/ha) |
|---|---|---|
| | **Herbicides** | |
| propanil | 1; January | 15 |
| bispyribac sodium | 1; January | 0.5 |
| clomazone | 1; January | 4.0 |
| quinclorac | 1; January | 6.0 |
| | **Fungicides** | |
| epoxiconazole | 2; March | 2.4 |
| difenocolazole | 2; March | 0.5 |
| azoxystrobin | 2; March | 0.5 |
| tebuconazole | 2; March | 1.2 |
| carbendazim | 2; March | 2.0 |
| isoprothiolane | 2; March | 2.5 |
| kresoxim-methyl | 2; March | 2.4 |
| trifloxystrobin | 1; March | 1.6 |
| tricyclazole | 2; March | 0.36 |
| | **Insecticides** | |
| thiamethoxam | 1; March | 0.36 |
| λ-cyhalothrin | 1; March | 0.36 |

The cultivation process was performed using the cropping system developed in Uruguay. Tillage was performed in October−November. Clomazone and glyphosate were sprayed before seeding, and a single fertilizer dose of 100 kg/ha was applied. Heavy rainfall in November (619 mm, late October−November) forced the seeding to be conducted in mid-December, using an amount of 161 kg seed/ha. The rice variety used was El Paso L144.[17] The emergence of rice began on December 22, and a week after quinclorac was applied. Finally, on January 17th bispyribac-sodium, clomazone, and quinclorac were applied. Rainfalls at the end of January and February were again very intense, 493 mm (for a complete rainfall calendar during the cropping season, see Figure S1-SM in the Supporting Information). Fungicides listed in Table 1 were sprayed twice in March along with the insecticides thiamethoxam and λ-cyhalothrin.

All of the pesticide applications were made using a 10 L rucksack to avoid contamination of the rest of the crop due to spray drift. The application of such a massive pesticide application has been done

**Table 2. Chemical Structures of the Pesticides with Their Corresponding MRLs and Status in the European Union and United States (US)**

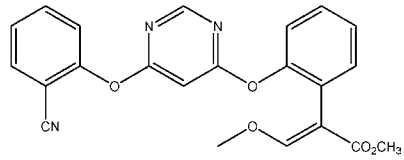
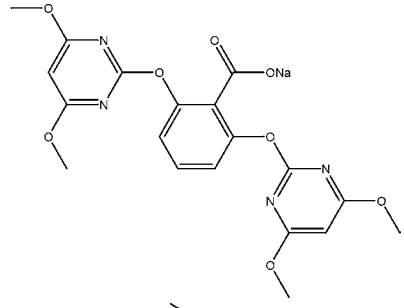
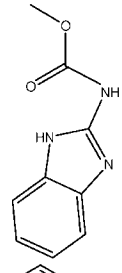
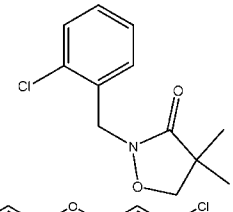
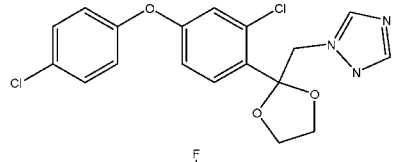
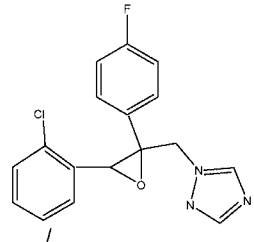
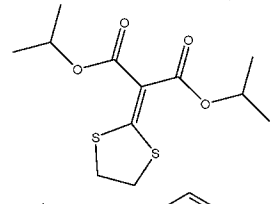
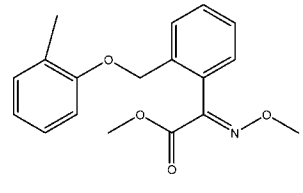
| Pesticide name | MRL (mg/kg) Reg. (EC) No 396/2005 | Status under Directive 91/414/EEC | MRL (mg/kg) US | Chemical Structure |
|---|---|---|---|---|
| Azoxystrobin | 5.0 | Included | 5.0 |  |
| Bispyribac sodium | --- | Included | 0.02 |  |
| Carbendazim | 0.01 | Included | --- |  |
| Clomazone | 0.01 | Included | 0.02 |  |
| Difenoconazole | 0.05 | Included | --- |  |
| Epoxiconazole | 0.1 | Included | --- |  |
| Isoprothiolane | 0.01 | Not included | --- |  |
| Kresoxim methyl | 0.05 | Included | --- |  |


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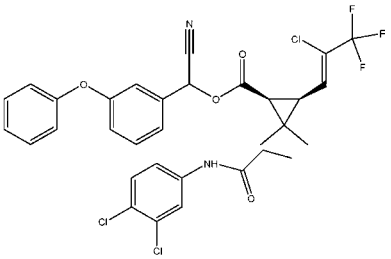
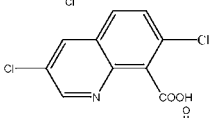
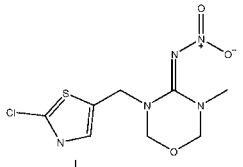
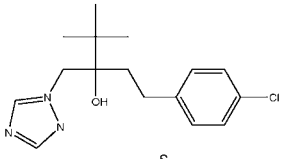
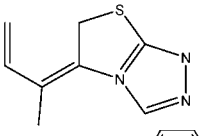
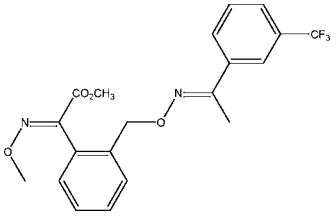
**Table 2. continued**

| Pesticide name | MRL (mg/kg) Reg. (EC) No 396/2005 | Status under Directive 91/414/EEC | MRL (mg/kg) US | Chemical Structure |
|---|---|---|---|---|
| λ- cyhalothrin | 1 | Included | 1 |  |
| Propanil | 0.2 | Not included | 10 |  |
| Quinclorac | 5 | Not included | 5 |  |
| Thiamethoxam | 0.05 | Included | 0.02 |  |
| Tebuconazole | 2.0 | Included | --- |  |
| Tricyclazole | 1.0 | Not included | --- |  |
| Trifloxystrobin | 0.02 | Included | 3.5 |  |

exclusively for the present study to ensure pesticide residues in the rice. In normal cropping conditions, only two pre-emergence herbicides (glyphosate and clomazone), a postemergence herbicide, one or two fungicides, and one insecticide if needed are used during the whole cultivation cycle.

Rice was harvested in May, and no postharvest treatment was performed. The soil of the crop plot where the study was conducted was removed, aerated, and covered with normal rice straw. The parcel was kept fallow for the next cropping season, and it will start to be prepared to be used in October (spring) 2012.

After harvest, rough rice was cleaned, dried to 13% humidity, and homogenized to obtain 100 kg of paddy rice, which was then further processed to yield brown, white, and broken rice and rice bran. Homogenized samples of each commodity were taken following standard procedures, and afterward they were transferred to the laboratory.

*Preparation of the "Blank" Test Material.* The grain rice used for blank test material was cropped and harvested in another parcel under similar conditions but without any pesticide treatment in the field, 1000 m away from the field where the rice for this study was cropped. After harvest, the rice grain was processed under the same conditions as described under Preparation of the Treated Material.

**Laboratory Experiments.** *Sample Preparation.* Once the samples arrived at the laboratory, a representative subsampling was performed.[18] All of the subsamples (500 g) were kept for 24 h in a desiccator over silica gel blue (Merck) before being milled in a cereal grain mill purchased from SAMAP (Andolsheim, France) to obtain the flour fractions.

The samples were extracted using citrate-buffered QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) and original QuEChERS described in our previous work.[2] QuEChERS is based on a salting-out extraction with acetonitrile and different salts ($MgSO_4$ and NaCl in the case of the original method and citrate salts for the citrate-buffered version), followed by a dispersive cleanup method with $MgSO_4$ and certain adsorbents (C-18, PSA).[19] In this case the extraction step was performed on 5 g of sample, which was extracted with 10 mL of ultrapure water and 15 mL of acetonitrile with the appropriate salts according to the method. The shaking step was performed using an automatic axial extractor (AGYTAX, Cirta Lab. S.L., Spain) for 16 min instead of the traditional manual shaking. Then two different cleanup steps were performed; the citrate-buffered version comprised the use of $MgSO_4$, C-18, and PSA, whereas for the original method only PSA and $MgSO_4$ were used.

*Recovery Studies.* For the recovery studies, a representative portion of a homogenized rice milled sample was weighed and transferred to a glass mortar. Five replicates were fortified homogeneously with a standard solution in acetone to reach 20 and 200 μg/kg of the studied pesticides, respectively. The mixture was then gently blended in the mortar for 30 min, to assess the homogeneity of the sample. The sample was allowed to stand at room temperature overnight, until its extraction with the different QuEChERS methods and LC or GC analysis.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## RESULTS AND DISCUSSION

**Target Compound Selection.** The pesticides with their MRLs from the EU and United States are shown in Table 2. These pesticides were selected on the basis of their use in rice production in Uruguay.

Although since 2009, the Uruguayan rice Good Agricultural Practices guidelines[3] have prohibited the use of the fungicide carbendazim, it was selected for this study because in the EU it can be used in rice crops.

**Optimization of LC-MS/MS and GC-MS Conditions.** The instrumental optimization of the compounds included in the LC method was made by FIA of the individual standard solutions at a concentration of 1 mg/L in methanol. In this process the precursor and the product ions were chosen, along with the optimum fragmentation voltage for the precursor ion and the collision energies for the product ions. The transitions of the most abundant productions (SRM1) were used for quantitation and the second ones in abundance (SRM2) for identification. The protonated and deprotonated molecules ($[M + H]^+$ and $[M - H]^-$) were selected in all cases as the precursor ions as they presented the highest abundance (Table 1). Afterward, in the product ion scan mode, two product ions for each compound were selected, along with their corresponding optimum collision energies. Tebuconazole ($m/z$ 70.0) and clomazone ($m/z$ 89.0) yielded low-mass ions. Obtaining such low masses represents a disadvantage as it entails a decrease in specificity. Nevertheless, ions were chosen for product ions as no other higher-mass ions were sensitive enough. Despite the fact that kresoxim-methyl and difenoconazole are LC amenable compounds, these pesticides presented sensitivity problems and could not be detected properly by liquid chromatography; therefore, they were included in the GC-MS method.

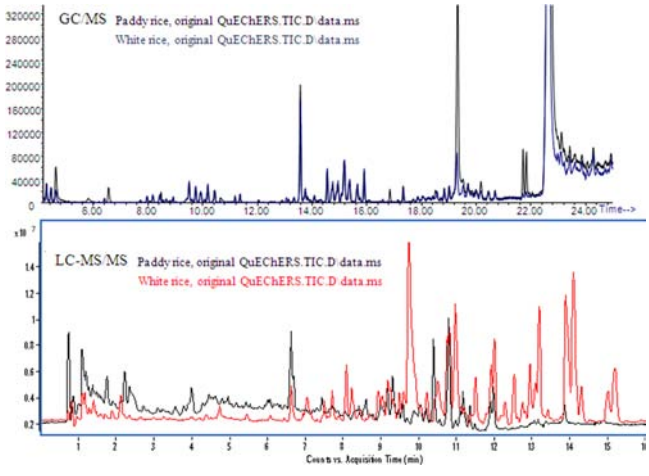


**Figure 2.** Total ion chromatograms (TIC) obtained from the (bottom) LC-QqQ/MS and (top) GC-MS analysis of 10 μg/kg spiked level in white rice and paddy rice.

Figure 2 shows the total ion chromatogram (TIC) in full-scan mode obtained by GC-MS and LC-ToF/MS for paddy and white rice extracted with the original QuEChERS method.

The GC-MS analyses were performed in SIM mode. Three ions were used for identification criteria according to DG SANCO document criteria.[18] The most abundant ion was used for quantitation and the other two as qualifiers. The selected ions along with their relative abundances and the typical retention times are summarized in Table S2 of the Supporting Information. Response factors were calculated using matrix-matched and solvent-only calibrations.

**Method Validation.** In a previous work[2] we compared the performances of four different methodologies based on the QuEChERS method (original QuEChERS, citrate QuEChERS with and without cleanup, and acetate-buffered QuEChERS) for the analysis of 54 pesticides in white rice by LC-MS/MS. In this study some new pesticides, difenoconazole, quinclorac, isoprothiolane, and λ-cyhalothrin, were added, and the performances of these methods were evaluated for a paddy rice matrix to decide which method would be appropriate for the analysis of the other commodities. The original QuEChERS and the citrate-buffered version presented the best performance; thus, they were selected for the analysis of the different commodities. Both methods were validated according to the European requirements and guidelines[18] and performed by considering accuracy (recovery experiments), precision, selectivity, linearity, and the limits of detection (LOD) and quantification (LOQ) of each of the pesticides under study.

Accuracy and repeatability expressed as relative standard deviation (RSD) were evaluated by LC and GC using fortified blank samples of the two representative commodities, white and paddy rice, at two concentration levels (20 and 200 μg/kg, $n = 5$), obtaining satisfactory recoveries except for quinclorac, which could not be determined with any of the studied methods, as depicted in Table 3.

For the LC experiments, at 20 μg/kg, the RSDs were in the ranges of 0.3−16 and 1−21% for white and paddy rice, respectively.

The RSDs obtained after the GC analyses were below 5 and 20% for white and paddy rice, respectively.

The LODs in the LC and GC experiments were determined as the lowest concentration of each analyte in which the confirmation transition or ion presented a signal-to-noise ratio (S/N) of 3:1. At this concentration the S/N of the quantification signal was calculated; if this value was >10, then it was settled as the LOQ. Otherwise, a higher concentration of the quantification signal was used for the calculation of the LOQ. Both the LOD and LOQ were specified at a concentration level that ensured compliance with the European Commission criteria for quantitative residue methods.[18]

The LODs of the pesticides determined by LC-MS/MS were established in both matrices, at 5 μg/kg, for all pesticides, whereas the LODs of the GC amenable pesticides were in the same order in both methods. Kresoxim-methyl presented the lowest LODs (11 μg/kg for both matrices), whereas λ-cyhalothrin and trifloxystrobin presented the highest ones, around 100 μg/kg for paddy rice. For white rice the LODs were 82 and 29 μg/kg, respectively. No interfering peaks appeared at the retention times of the compounds, thus demonstrating the selectivity of the analytical methods. Moreover, the linearity of each analyte was evaluated for both methods in the two representative commodities. All of the pesticides presented coefficients of variation >0.99 in both LC and GC experiments.

**Matrix Effects (ME).** Due to the complexity of the commodities under study, the ME was evaluated to determine if there were significant matrix interferences during the analysis. The ME was studied by comparison of the slopes of the calibration curves in solvent and in matrix. Signal enhancement occurs if the percentage of the difference between these slopes


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**Table 3. Recoveries and RSDs (Percent) of Fortified Pesticides from Rice versus Matrix-Matched Standard Calibration ($n$ = 5), Obtained by LC and GC Analyses**

| | white rice | | | | paddy rice | | | |
|---|---|---|---|---|---|---|---|---|
| | original QuEChERS | | citrate-buffered QuEChERS | | original QuEChERS | | citrate-buffered QuEChERS | |
| pesticide | 20 μg/kg | 200 μg/kg | 20 μg/kg | 200 μg/kg | 20 μg/kg | 200 μg/kg | 20 μg/kg | 200 μg/kg |
| azoxystrobin | 81 (9) | 99 (4) | 73 (2) | 112 (7) | 89 (7) | 101 (20) | 88 (2) | 103 (6) |
| byspiribac sodium | 83 (13) | 103 (6) | 127 (5) | 77 (13) | 102 (15) | 105 (9) | 92 (21) | 99 (17) |
| carbendazim | 77 (0.3) | 93 (3) | 70 (2) | 105 (6) | 94 (11) | 96 (9) | 100 (6) | 90 (4) |
| clomazone | 79 (4) | 95 (6) | 74 (2) | 108 (6) | 88 (9) | 115 (5) | 87 (7) | 102 (6) |
| difenoconazole[a] | 71 (15) | 124 (11) | 41 (10) | 72 (9) | 79 (7) | 61 (20) | 45 (6) | 67 (5) |
| epoxiconazole | 69 (7) | 101 (7) | 101 (10) | 121 (5) | 77 (11) | 69 (11) | 77 (11) | 67 (9) |
| isoprothiolane | 81 (1) | 79 (15) | 87 (7) | 117 (4) | 74 (5) | 71 (7) | 71 (12) | 96 (6) |
| kresoxim-methyl[a] | 94 (13) | 90 (10) | 115 (10) | 105 (9) | 90 (6) | 93 (5) | 107 (19) | 72 (6) |
| λ-cyhalothrin[a] | 97 (9) | 111 (9) | 100 (9) | 88 (10) | 87 (1) | 87 (2) | 91 (10) | 88 (6) |
| propanil | 72 (3) | 86 (4) | 111 (5) | 117 (2) | 87 (6) | 87 (15) | 81 (6) | 87 (8) |
| quinclorac | 44 (15) | 30 (3) | 52 (3) | 54 (16) | 31 (5) | 55 (10) | 48 (6) | nd[b] |
| tebuconazole | 74 (11) | 97 (5) | 78 (2) | 121 (7) | 67 (14) | 65 (11) | 77 (15) | 70 (8) |
| thiamethoxam | 95 (12) | 112 (3) | 81 (3) | 120 (6) | 83 (8) | 91 (7) | 101 (20) | 98 (7) |
| tricyclazole | 97 (5) | 98 (6) | 74 (2) | 99 (3) | 88 (10) | 86 (3) | 83 (7) | 86 (6) |
| trifloxystrobin[a] | 97 (10) | 115 (13) | 104 (13) | 102 (12) | 92 (4) | 97 (10) | 82 (4) | 86 (5) |

[a]Pesticides analyzed by GC-MS. [b]nd, not detected.

**Table 4. Matrix Effect of the Selected Pesticides for Paddy and White Rice Using Both Extraction Methods**

| | | white rice | | paddy rice | |
|---|---|---|---|---|---|
| chromatographic system | pesticide | ME (%) original QuEChERS | ME (%) citrate QuEChERS | ME (%) original QuEChERS | ME (%) citrate QuEChERS |
| GC-MS | difenoconazole | 9 | −4 | 19 | 14 |
| | kresoxim-methyl | 3 | −3 | −3 | −6 |
| | λ-cyhalothrin | 3 | −3 | 10 | 2 |
| | trifloxystrobin | 10 | −2 | 10 | 3 |
| LC-MS/MS | azoxystrobin | 25 | 10 | −10 | −27 |
| | bispyribac sodium | 8 | 6 | 15 | 10 |
| | carbendazim | 11 | 14 | −20 | −14 |
| | clomazone | 5 | 31 | 9 | −23 |
| | epoxiconazole | −11 | 5 | −38 | −50 |
| | isoprothiolane | −10 | −11 | −35 | −39 |
| | propanil | −7 | −9 | −62 | −29 |
| | quinclorac | 5 | 24 | −25 | −26 |
| | tebuconazole | −28 | −17 | −59 | −54 |
| | thiamethoxam | 2 | 22 | 0 | −13 |
| | tricyclazole | 1 | 3 | −15 | −26 |

**Table 5. Concentration Distribution of the Pesticide Residues Detected in the Different Commodities with Their Corresponding %RSD in Parentheses, LOQs, and $K_{ow}$ Values**

| extraction method | pesticide | LOQ | paddy rice (μg/kg) | brown rice (μg/kg) | rice bran (μg/kg) | white rice (μg/kg) | log $K_{ow}$ |
|---|---|---|---|---|---|---|---|
| original QuEChERS | azoxystrobin | 5 | 210 (7) | 9 (2) | 17 (3) | <LOQ | 2.5 |
| | carbendazim | 5 | 719 (9) | 81 (6) | 110 (6) | 12 (2) | 1.4 |
| | epoxiconazole | 8 | 431 (7) | 44 (3) | 10 (2) | 32 (2) | 3.3 |
| citrate QuEChERS | difenoconazole[a] | 50 | 108 | <LOQ | 155 | <LOQ | 4.4 |
| | isoprothiolane | 5 | 807 (8) | 656 (8) | 131 (4) | 153 (4) | 2.8 |
| | kresoxim-methyl[a] | 11 | 64 (3) | 66 (3) | <LOQ | <LOQ | 3.4 |
| | λ-cyhalothrin[a] | 250 | <LOQ | <LOQ | <LOQ | <LOQ | 7.0 |
| | tebuconazole | 5 | 774 (8) | 178 (3) | 6 (2) | 29 (2) | 3.7 |
| | thiamethoxam | 5 | 32 (2) | <LOQ | 20 (3) | <LOQ | −0.13 |
| | tricyclazole | 5 | 639 (10) | 34 (5) | 262 (8) | 9 (2) | 1.4 |
| | trifloxystrobin[a] | 250 | | <LOQ | | | 4.5 |

[a]Pesticides analyzed by GC-MS.


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Table 6. Range Mean Content (Percent) of Organic Fractions of Rough Rice and Its Milling Fractions at 14% Moisture

| chemical composition | paddy rice | brown rice | white rice | hull | rice bran |
|---|---|---|---|---|---|
| carbohydrates | 64−73 | 73−87 | 77−89 | 2−2.8 | 11.3−14.9 |
| proteins | 5.8−7.7 | 4.3−18.2 | 4.5−10.5 | 13.2 | 14.6 |
| crude ash | 2.9−5.2 | 1.0−1.5 | 0.3−0.8 | 13.2−21.0 | 6.6−9.9 |
| crude fat | 1.5−2.3 | 1.6−2.8 | 0.3−0.5 | 0.3−0.8 | 15−19.7 |
| crude fiber | 7.2−10.4 | 0.6−1.0 | 0.2−0.5 | 34.5−45.9 | 7.0−11.4 |

is positive. If it is negative, it is indicative of signal suppression. Depending on the value of this percentage, different MEs could be observed. A percentage between −20 and 20% was considered as no matrix effect, because this variation is close to the repeatability values. A medium matrix effect occurred when the values were between −50 and −20% or 20 and 50%, and a strong matrix effect would be below −50% or above 50%.[20]

As shown in Table 4, the pesticides analyzed by GC-MS did not present ME. In LC-MS/MS experiments, the signal suppression of paddy rice was more pronounced than in white rice. In paddy rice, azoxystrobin and isoprothiolane presented a medium ME, whereas propanil presented the highest signal suppression. In white rice, citrate QuEChERS presented a higher ME; nevertheless, this effect is almost neglible. Only cyhalofop-butyl, trifloxystrobin, and difenoconazole presented around 30% of signal suppression, whereas clomazone presented 31% of signal enhancement.

**Pesticide Distribution.** The distribution of the pesticide residues in the different commodities from processed rice is presented in Table 5 with their corresponding octanol−water partition coefficient values ($K_{ow}$).[21]

Pesticide distribution could be explained as a combination of many factors, such as matrix chemical composition, pesticide lipophilicity, and mode of action (systemic vs nonsystemic).[22] Table 6 shows the main components of these matrices.

None of the applied herbicides were present in the processed rice. This result is in accordance with the analysis of more than 20 real samples from Uruguay and Spain, where no herbicide residue was found.[2] However, Dors et al.[16] reported the presence of clomazone and bispyribac sodium in rice bran and paddy rice obtained after the processing of treated rice. A possible explanation could be the differences in the frequency of the applied doses, the rice variety, and differences in the irrigation system, as well as the weather conditions. Rainfalls were very intense after each herbicide treatment (631 mm in late January/February 2010), and they could have washed the herbicides or produced their leaching from the rice plant (Supporting Information, Figure S1).

Different fungicides were detected in most of the selected commodities. The pesticides belonging to the triazole group, epoxiconazole, tebuconazole, and tricyclazole, were found in all of the commodities, whereas difenoconazole and the strobilurins (azoxystrobin, kresoxim-methyl, and trifloxystrobin) were detected mainly in the most lipophilic samples, paddy, bran, and brown rice. A possible explanation could be that these pesticides are the most lipophilic compounds of this group (high $K_{ow}$ values); thus, their distribution on these high fat content matrices is reasonable (see Table 6).

Other fungicides such as isoprothiolane and carbendazim were also found in all pf the matrices. Trifloxystrobin and λ-cyhalothrin were below the LOQ.

As shown in Table 5, polishing causes a reduction in the pesticide residue concentration along the production chain. Paddy rice presented the highest amount and number of pesticide residues, whereas the lowest amounts of pesticides were found in white rice. A possible assumption is that the difference between the amounts of pesticide residues found in paddy and brown rice should have remained in the hull, but this was not the general trend. Amounts of 5−20% from the original amounts found in paddy rice of seven pesticides (azoxystrobin, carbendazim, difenoconazole, epoxiconazole, thiametoxam, tricyclazole, tebuconazol) were detected in brown rice, but for kresoxim-methyl almost the same amounts were found in paddy and brown rice, indicating that this pesticide is not concentrated in the hulls according to its systemic mode of action. During the milling process of brown rice, rice bran and white rice are obtained. It could be considered that the sum of the pesticide residues in brown rice and rice bran should be the same as the total amount found in brown rice, but this was not the case for most of the pesticides under study. Pesticide residues in bran and white rice are much lower than the theoretical ones if a processing factor of 1 is assumed for their distribution. From the pesticides under study, only isoprothiolane was distributed evenly between paddy rice, brown rice, and rice bran, but it did not follow the same trend in polished rice. In this case, the amount of isoprothiolane residues in brown rice is 80% of the amount present in paddy rice, and the corresponding 15% of the original amount was detected in bran rice. Nevertheless, in polished rice only a fifth of the expected theoretical amount of isoprothiolane was found (Tables 5 and 6).

The most used azole fungicides, tebuconacole and epoxiconazole, were found in polished rice, whereas difenoconazole and tricyclazole were concentrated in rice bran. Other pesticides did not follow such a direct relationship. As this is a single experiment, the data are not sufficient to calculate the processing factors of the pesticide residues, but it can be assumed that these factors should be determining for the residues occurrence, especially for white rice. These results also suggest that dust evolved during brown rice processing could contain the remaining undetected portion of the pesticides. If this situation is confirmed, mill worker exposure to contaminated dust should be also taken into account.

At the applied doses, rice hull removal diminished the residues of the pesticides used in rice cultivation. However, the residue levels of the pesticides found in the different commodities were below the corresponding MRLs established by the EU for rice except for isoprothionale and carbendazim.

In conclusion, the present study demonstrates the importance of evaluating the fate of pesticides and its residues from the field to the final food or feed. Many results shown in the present work are nonpredictable a priori due to the myriad factors (lipophicity, mode of action, p$K_a$, among others) that influence their occurrence and distribution in the rice grain.

## ■ ASSOCIATED CONTENT

### Supporting Information

Table S1.Values of the instrumental settings optimized for each compound in LC-QqQ/MS used for pesticide confirmation: precursor ion, fragmentor voltage (V), product ions and their collision energies (CE), ionization mode and retention times ($R_t$). Table S2. Chromatographic conditions for pesticide confirmation in GC-MS. [a]Quantitation ions in bold letters. Figure S1. Average amount of rainfalls during rice cropping season 2009−2010. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*E-mail: amadeo@ual.es. Phone: +34950015034. Fax: +34950015483.

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### Notes

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