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Critical Revision and Development Perspectives of Herbicide Residues Analysis in Agro Ecosystems

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1. Introduction

Weeds are defined as plants growing in the inadequate place at the inadequate moment. They interfere with the normal crop development and can drop down yields dramatically. Winter cereals show lower losses in average, due to its dense stands and competitive abilities. But, in summer crops, particularly corn that has low densities per area and lower initial growth rates than weeds, very high yield reductions are possible. Rice is very susceptible to weed interferences. There are evidences of production losses from 20 to 96% (Table 1). Weed control is performed in 100% of rice cultures either manually, mechanically or chemically. In average, registered losses are around 10% of production even when weed control has been done. Weed control cost is around 5%. Consequently, the global cost of this factor is around 15% of production. In sum, this 15% is the negative impact weeds have in agricultural and food production.
To control losses in the crop production and as support of the new agronomical techniques for intensive cropping, like no tillage or direct sewing techniques, weed chemical control has been adopted as a usual technique to improve yields.

2. Herbicide application

Herbicide application timing depends on various factors such as class of herbicide, weed species aiming to control, mechanic labor, climate, conditions and soil type. This latter factor is very important, particularly soil preparation, previous and present vegetation, humidity content on soil surface and temperature that makes probable a quick weed germination. A

Crop	% Production Decrease	Cause	Reference
Rice	20-30	Competition, inadequate weed control. (Brazil)	(Harri, L., 1994)
Rice	25 (11pl/m^2) 49 (54 pl/m^2) 79(269 pl/m^2)	*Echinocloa colonum*	(Smith, R. J., 1968)
Rice	40	Competition, weed control 45 d post crop emergence.	(Chebatarrof N., 2007)
Corn	10-15	Weed resistant to control	(Cepeda, S. et al., 1995)
Corn	27-40 97 (if the weed is *Sorghum halepense* or *Cynodon dactylon*)	No weed control at all	(Rossi, A. et al., 1996)

Table 1. Losses caused by weeds in rice and corn.

general concept is that the herbicide should be applied when the crop has the maximum resistance and weeds are in the most susceptible period (Genta and Villamil, 1992). Herbicide application at pre-emergence stage is closely related with the culture labors of the soil. Once the crop has been sewed, herbicide application is related with crop development and how infested are the present weeds.

2.1 Preplant treatment

In rice crops, an herbicide application (i.e. glyphosate) is usually applied in autumn to control existent weeds when machinery labor is needed. This is a non selective treatment and it is done in order to prepare the surface for future work such as levees for irrigation. Probability of weed germination increases with earthwork, favorable conditions of humidity and crop absence.

After land planting, due to humidity conditions of soil in autumn and winter, often great amounts of weeds appear and a herbicide is applied to control them (i.e. glyphosate).

2.2 Preemergence treatment

This treatment is done after sowing but before weed emergence. The control consists in combating the seeds that could be in the first soil layer or seeds that have recently germinated when a good control can be achieved.

The main advantage of pre-emergence and preplant treatments is eliminating weed competition in the initial steps of crop development. In general, these treatments are safer and more consistent than post-emergence ones because in crops that irrigation is needed or when it has rained it is difficult to apply the product or hoe. Some disadvantages are that perennial weeds are hard to control and the effectiveness of the products depends on the kind of soil (organic matter content and pH). Also these products require to be mobilized by water in soil, so they are not effective under drought conditions.

2.3 Postemergence treatment

Selective products must be used in order to eliminate weeds without damaging the crop. The class of weed that can be controlled depends on the tolerance of the crop to the

herbicide. The risk of damaging the crop is high; temperature is one of the factors that affects the most the effectiveness and volatilization of many post-emergent herbicides, like clomazone. In general when they are applied weeds have already born. An effective weed control is sometimes difficult to achieve because there are many factors that must be taken into account (right climatic conditions, doses, soil humidity level, etc.)

Herbicides are the most employed agrochemicals in agriculture nowadays. Pre or post emergence, root or leaf absorbed, they are applied in huge amounts to hamper weed development worldwide. There are many possible molecular targets to exert the herbicidal action and therefore, herbicides are the vastest group of agrochemicals as there are 53 different chemical families of weed controllers. The most representative herbicide chemical classes are listed in Table 2.

GROUP OF HERBICIDES	EXAMPLES	MODE OF ACTION	PERSISTANCE IN SOIL (DT50)*
Amide	Florasulam	Branched chain amino acid synthesis (AHAS or ALS) inhibitors	2 - 18 d
	Propanil	Photosynthetic electron transport inhibitor	N/A
Aromatic Acid	Dicamba Quinclorac	Auxin growth regulator	14 d N/A
	Bispyribac-sodium	Branched chain amino acid synthesis (AHAS or ALS) inhibitors	< 10 d
	Aminopyralid Picloram	Auxin growth regulator	8 – 35 d 30 – 90 d
Bipyridiles	Paraquat	Promotes autoxidation	
Dinitroaniline	Pendimethalin	Microtubule assembly inhibition	3 - 4 mo
Diphenyl Ether	Oxyfluorfen	Protoporphyrinogen oxidase inhibitor	5 – 55 d
Imidazolinone	Imazapyr	Branched chain fatty acid biosynthesis inhibitors	30-150 d
organphosphate	Glyphosate	Inhibits 5-enolpyruvylshikimate-3-phosphate synthase.	1 - 130 d
Oxazole Herbicides	Topramezone	p-Hydroxyphenyl pyruvate dioxygenase inhibitor	9 – 81 d
Phenoxy Herbicides	MCPA 2,4-D Mecoprop-p Dichlorprop-P 2,4-DB	Auxin growth regulator	< 7 d < 7 d 3 - 13 d < 7 d
	Fenoxaprop-P-ethyl Cyhalofop-butyl Diclofop-methyl	Lipid biosynthesis inhibitors	1 – 10 d 2 – 10 h 1 – 57 d

GROUP OF HERBICIDES	EXAMPLES	MODE OF ACTION	PERSISTANCE IN SOIL (DT50)*
Pyrazole Herbicides	Pinoxaden	Lipid biosynthesis inhibitors	< 1 d
Pyridine Herbicides	Fluroxypyr	Auxin growth regulator	5 – 9 d
	Diflufenican		36,3 d
	Pyroxsulam	Branched chain amino acid synthesis (AHAS or ALS) inhibitors	< 15 d
Triazine Herbicides	Atrazine	Photosynthetic electron transport inhibitor	16 – 77 d
Triazolone Herbicides	Carfentrazone-ethyl	Branched chain amino acid synthesis (AHAS or ALS) inhibitors	2,5 – 4 d
	Propoxycarbazone-sodium	Branched chain amino acid synthesis (AHAS or ALS) inhibitors	12 - 56 d
	Flucarbazone-sodium		17 d
Triazolopyrimidine Herbicides	Flumetsulam	Branched chain amino acid synthesis (AHAS or ALS) inhibitors	≤ 1 mo
Urea	Isoproturon	Photosynthetic electron transport inhibitor	6-28 d
Sulphonylureas	Amidosulfuron	Branched chain amino acid synthesis (AHAS or ALS) inhibitors	3 – 29 d
	Bensulfuron-methyl		14 d
	Flupyrsulfuron-methyl-sodium		1 – 5 d
	Iodosulfuron-methyl-sodium		52 d
	Metsulfuron-methyl		10 – 21 d
	Pyrazosulfuron-ethyl		11 – 47 d
	Sulfosulfuron		1 – 7 d
	Thifensulfuron-methyl		19 d
	Triasulfuron		3,5 - 5,1 d
	Tribenuron-methyl		1,5 – 9,4 d
	Foramsulfuron		4 – 6 w
	Chlorsulfuron		
Unclassified Herbicides	Bentazone	Photosynthesis inhibition	12 d
	Clomazone	Carotenoid biosythesis inhibition	30 – 135 d

* Degradation half-life: d – days, w – weeks, mo – months; N/A: not available

Table 2. Most representative chemical families of herbicides

Economical aspects

Higher land, use intensity and short crop rotations (or sometimes no rotation at all) plus no tillage technology leads to an increase in the usage and dependency of agrochemicals. Pests and weeds problem increases when land use is more and more intensive. Weeds negatively affect grain production and quality, which declines prices. Also, harvesting becomes complex and more expensive. Resources competition and allelopathy interferences, like light, water, CO_2 and soil nutrient competition provokes important or complete economic losses. On top of that, weeds are pest hosts and disseminate crop infections.

2.4 Herbicides trade and usage

In 2009, herbicides represented 75% of agrochemical imports in kg and 59.5% in U$S/CIF, showing they continue to be the most employed agrochemicals in Uruguay. As shown in Figure 1, herbicides prevail in agrochemicals world trade. In Uruguay, this tendency is even stronger when compared to Brazil although Argentina has bigger differences than Uruguay (63%, 20% and 9% herbicides, insecticides and fungicides respectively) (casafe website).

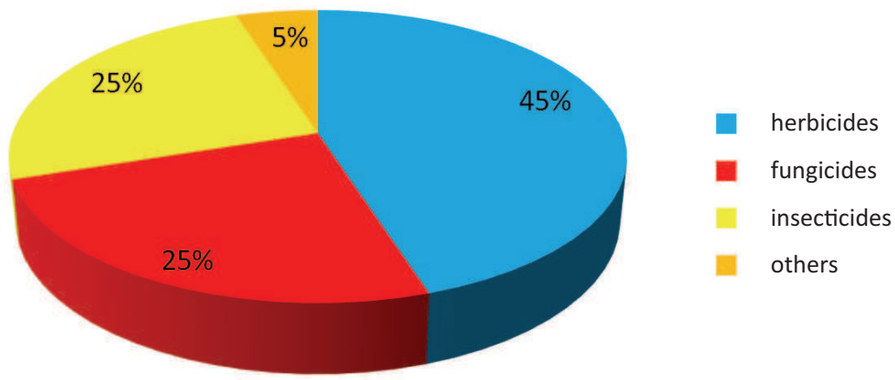

Fig. 1. Agrochemicals world trade (Rana, S. AGROW, 2010)

Herbicides show the higher relative increase when compared to fungicides and insecticides between 2003 and 2009. Glyphosate accounts for 57% of herbicides total.

As stated above, rice production needs intensive herbicide use. From 141.500 ha of rice crop analyzed and registered, the 98% (138.000 ha) required the appliance of herbicides with repeated control treatments in about the 10% of the treated area (Molina et al., 2010). Fields never used for rice crops were included in the area under treatment, along with pasture, which despite of doing the adequate rotation, showed the presence of weeds in quantities above the threshold for economic damage requiring control with herbicides. The active ingredients used are shown in Table 3.

As shown in Table 4, the most common herbicide treatment is atrazine for corn and sorghum in mixture with a chloroacetamide in pre-emergence. Post-emergent options such as imidazolinones (Imazethapyr + imazapyr or imazapic + imazapyr) are preferred for corn with zero tillage because of straw interactions with pre-emergent herbicides. However, at present there are only limited numbers of cultivars with tolerance for these herbicides and so very few hectares had been treated with imidazolinones in the last years. Other post-emergent options as growth regulators herbicides, the mixture of iodosulfuron plus foramsulfuron or topramezone had not been extensively adopted. They are more expensive

Products or mixtures applied	Area of appliance (ha) T	%
Quinclorac+Clomazone+Propanil	45660	30
Clomazone+Glyphosate	18198	12
Clomazone+Quinclorac	8975	6
Bispyribac-sodium+Clomazone	7521	5
Quinclorac+Clomazone+Propanil+Pyrazosulfuron-ethyl	6964	5
Bispyribac-sodium	5342	4
Clomazone+Propanil	4977	3
Others	52667	35
Total	150.304	100
Clomazone alone or as mixture	118017	79
Quinclorac alone or as mixture	77683	52

Table 3. Active ingredients used in rice production in Uruguay

Crop	Herbicide	Application
Corn	Atrazine	Pre-emergence
	chloroacetamides	
	Iodosulfuron + foramsulfuron	Post-emergence
	Topramezone	
	imidazolinonas	
Sorghum	Atrazine	Pre-emergence
	chloroacetamide	
Soybean	Glyphosate	Post-emergence
	Imazethapyr	pre/ post-emergence
	Diclosulam	pre/ post-emergence
Wheat and Barley	Pyroxsulam (only wheat)	Post-emergence
	Flucarbazone (only wheat)	Post-emergence
	Iodosulfuron	Post-emergence
	Pinoxaden	Post-emergence
	Diclofop-methyl	Post-emergence
	Fenoxaprop	Post-emergence
	Metsulfuron	Post-emergence
	Metsulfuron + Chlorsulfuron	Post-emergence
	growth regulators (2,4D amine, Dicamba, Picloram)	Post-emergence
Artificial pastures	Flumetsulam	Post-emergence
	2,4 DB ester	Post-emergence

Table 4. Herbicides most commonly used in extensive crops and artificial pastures in Uruguay

and their performances had not demonstrated clear advantages compared with the classic pre-emergent mixture of atrazine+chloroacetamides except in specific weed situations.

There are potential risks of persistence with this treatment including atrazine. In production systems alternating crops and pastures where corn or sorghum crops grown for silage are followed by pastures with legumes and grasses or by oats, plant-back period may resulted insufficient for atrazine dissipation and damage occurrence.

Soybean area, entirely GMO planted crop, is basically treated with glyphosate. Nevertheless, It has been registered an increased trend to complement glyphosate with residuals herbicides like imazethapyr or diclosulam looking to reduce number of glyphosate applications and to broad control spectrum. There is an increasing movement of the big breeders companies towards the inclusion in the new varieties, some resistant genes against particular herbicide mode of action. Following the trend initiated by the glyphosate resistant RR soybean, crop varieties resistant to imidazolinones (rice, corn) glufosinate (rice) sulphonylureas (sunflower) has been registered. The use of these varieties will boost the use of the specific herbicides and therefore new challenges on environmental risk assessment on soil, water and food can be foreseen.

Herbicide options for winter cereals, wheat and barley are various and especially in case of weed grasses. Number and total use of graminicides has increased markedly. The most used are pinoxaden, pyroxsulam, iodosulfuron, flucarbazone, diclofop methyl and fenoxaprop. The use of these herbicides has five-folded since 2006 even though winter crop area has just doubled during this period, in association with the widespread infestations of grasses in agriculture lands.

Other herbicides widely used for winter cereals are the sulphonylureas, metsulfuron and chlorsulfuron as they have a broad spectrum of weed control, may be sprayed early and have important residual effects, so satisfactory controls are reached in species with continued emergences fluxes as commonly happens with winter weed species in our country. However, application of these herbicides implies a risk of persistence as commonly happens when used in fallow seed-bed preparation without considering the plant-back guidelines for crop rotation. See for example Bradford et al, 2008. Its low cost and wide spectrum makes them highly attractive for use in mixtures with glyphosate in fallow seed-bed preparation for summer and winter crops, being frequent situations of persistence problems.

These problems, occasional phytotoxicity effects and risk of resistance, have been promoting a return to traditional growth regulators treatments such as 2,4-D amine in mixtures with dicamba, picloram, etc. or new options like aminopyralid. Also in artificial pastures use of herbicides is incremental. The most common treatment in this areas is flumetsulam alone or in mixtures with 2,4-DB ester.

In this introduction it is shown that herbicide usage has been integrated systematically in cropping systems, that there is no universal procedure to perform weed control and different herbicidal combinations are employed in different stages of crop production. Although some herbicides are used in very low doses, and many of them are designed to inhibit specific pathways of plant metabolism, their interference with metabolic pathways of other organisms is a growing concern. Some herbicides are persistent between two cropping systems and can affect the new crop, notably atrazine and the sulphonylureas. Therefore, for public health reasons and systems sustainability the knowledge of the level of remaining herbicide residues has to be ascertained. In the past, sometimes the low dosage of these compounds hampered a clear determination of their residual level in the different

environmental compartments. Nowadays the development of new sample treatment procedures either instrumental, like ASE or MASE or not, like QuEChERS, coupled to hyphenated MS/MS techniques allow the detection of very low levels of these compounds in different matrices. The present chapter aims to present some characteristics of herbicide analysis, to explore the reasons why many currently used herbicides have not been included in the most common Multi Residue Methods developed and to give some insights on the different aspects that should be considered when trying to include herbicides in a multiresidue method.

3. Herbicide residues analysis

The analysis of herbicides has been confined to single residue methods, or class herbicide residue analysis (phenoxyacid herbicides, imidazolinones, sulphonylureas, triazines). Nevertheless, the real situation is that cationic, anionic, basic or acid herbicides together with fungicides and insecticides can be applied successively over a crop looking for different protecting effects. They accumulate in the crop and the environment and when evaluating their presence in the different compartments they can seldom be determined in one single analytical procedure. To define the scope of a multirresidue method that will allow the simultaneous determination of many herbicides belonging to different chemical classes, the physicochemical properties of the agrochemicals must be carefully evaluated. The physicochemical properties of some representative herbicides are listed in Table 5.

Herbicide	Koc	log Kow	log Ws (mg L^{-1}) 20-25°C	H (Pa m^3 mol^{-1})	Vapor Pressure (mPa)
2,4-D	60	2.58 -2.83	4.37	1.32 x 10-5	1.86 x 10-2
2,4-DB	-	-	1.66	-	-
Amidosulfuron	-	1.63	0.95	5.34 x 10-4	2.20 x 10-2
Aminopyralid	-	0.201	3.39	-	9.52 x 10-6
Atrazine	39 – 173	2.50	1.52	1.5o x 10-4	3.85 x 10-2
Bensulfuron-methyl	-	0.79	1.83	2.00 x 10-11	2.80 x 10-9
Bentazone	13.3 – 176	-0.46	2.76	-	5.4 x 10-3
Bispyribac-sodium	-	-1.03	4.87	3.12 x 10-11	5.05 x 10-6
Carfentrazone-ethyl	15 – 35	3.36	1.08	2.47 x 10-4	7.2 x 10-3
Chlorsulfuron	40	-0.99	4.50	3.50 x 10-11	3.00 x 10-6
Clomazone	150 – 526	2.50	3.04	4.19 x 10-3	19.2
Cyhalofop-butyl	5247	3.31	-0.36	9.51 x 10-4	5.30 x 10-2
Dicamba	2	-1.88	> 5.40	6.10 x 10-5	1.67
Dichlorprop-P	-	-0.25	2.77	2.47 x 10-5	0.06
Diclofop-methyl	14000 - 24400	4.58	-0.10	2.19 x 10-1	0.25
Diflufenican	-	4.90	-1.30	1.18 x 10-2	4.25 x 10-3
Fenoxaprop-P-ethyl	-	4.58	-0.15	2.74 x 10-4	1.80 x 10-1

Florasulam	2-069	-1.22	3.80	4.35 x 10-7	1.00 x 10-2
Flucarbazone-sodium	-	-1.84	4.64	< 1 x 10-11	< 1 x 10-6
Flumetsulam	5-182	-0.68	1.69	-	3.7 x 10-7
Flupyrsulfuron-methyl-sodium	-	0.10	2.78	1 x 10-8	1 x 10-6
Fluroxypyr	-	-1.24	3.76	1.06 x 10-8	3.78 x 10-6
Foramsulfuron	38 – 151	-0.78	3.52	5.80 x 10-12	4.20 x 10-8
Glyphosate	-	< -3.20	4.02	< 2.10 x 10-7	1.31 x 10-2
Imazapic	-	0.39	3.33	-	< 1 x 10-2
Imazapyr	-	0.11	4.05	-	< 0.013
Imazaquin	-	0.34	1.78-2.08	3.70 x 10-12	< 0.013
Iodosulfuron-methyl-sodium	0.80 – 152	-0.70	4.40	2.29 x 10-11	6.70 x 10-6
Isoproturon	-	2.50	1.81	1.46 x 10-5	3.15 x 10-3
MCPA	-	2.75	2.47	5.50 x 10-5	2.30 x 10-2
Mecoprop-p	12-025	0.1004	2.94	2.18 x 10-4	1.60
Metsulfuron-methyl	-	0.018	3.45	4.50 x 10-11	3.3 x 10-7
Oxyfluorfen	2891 (sand)	4.47	-0.94	-	0.0267
Pendimethalin	-	5.20	-0.48	2.728	1.94
Picloram	-	1.90	-0.25	-	8 x 10-11
Pinoxaden	-	3.20	2.30	-	2 x 10-9
Propanil	239 – 800	3.30	2.11	1.70 x 10-4	0.02
Propoxycarbazone-sodium	28,8	-1.55	4.62	< 1 x 10-10	< 1 x 10-5
Pyrazosulfuron-ethyl	-	3.16	0.99	-	4.20 x 10-5
Quinclorac	-	-0.74	-1.19	-	< 0.01
Sulfosulfuron	33	-0.77	3.21	8.83 x 10-9	3.1 x 10-5
Thifensulfuron-methyl	-	0.02	3.35	9.70 x 10-16	1.70 x 10-5
Topramezone	-	-1.52	2.71	-	< 1 x 10 -12
Triasulfuron	-	-0.59	2.91	< 8 x 10 -5	< 2 x 10 -3
Tribenuron-methyl	-	0.78	3.31	1.03 x 10-8	5.20 x 10-5

Table 5. Physicochemical and environmental properties of some selected herbicides

As an example, when looking for a procedure that can include the analysis of imidazolinone residues their peculiar pH behavior has to be considered. The Imidazolinones are amphoteric molecules that can exist in anionic, neutral or cationic states depending upon the pH of the environment. The pKa values of imidazolinone herbicides range from 1.9 to 3.9. When the pH of the extracting solvent is greater than their pKa, these herbicides are usually present mainly in an anionic state as carboxylates. On the other hand, the acidic functional group is completely in a non-ionized state when the pH is two units lower than the most

acidic pKa value. Therefore factors such as pH, organic carbon content, and ionic strength may affect their extractability to perform the analysis. All these factors also affect the fate of the imidazolinone herbicides in the environment which are relatively persistent in soil with half-lives ranging from 30 to 150 days and therefore, may have carryover effects on the rotation system of winter-summer crops. Moreover, it has been reported that the imidazolinone herbicides show high potential for leaching because of their relatively low pKa values (1.9–3.9). (Ramezani et al., 2009)

But not only have these factors operated. The matrix characteristics are crucial for the precision and accuracy of the analysis. Differences in pesticide absorption and recoveries were found when comparing results from contrasting soils (Boivin et al., 2005).

Environmental samples

The major public health concern on herbicide residues in the environment is due to the fact that many of them have low Koc and relatively low Kow and therefore, can either lixiviate, leach or run off and contaminate ground and surface water. Many herbicides have been identified as endocrine disrupters and they threaten wild life as Atrazine (Barchanska & Baranowska, 2009). The usual methodology for contaminant analysis in water is the solid phase extraction either off line or on line coupled with the analytical instrument (GC or HPLC). The adsorbed compounds are eluted from the stationary phase with a polar organic solvent and the extract is analyzed directly. This simple procedure allows sample concentration increasing the limit of detection of the method to $ngkg^{-1}$ level or ppts, fulfilling the requirements of the European legislation on drinking water, where pesticide limits of quantification (LOQs) of $0.025\mu gL^{-1}$, four times lower than the maximum allowed ($0.1\mu gL^{-1}$), are required. To perform the SPE extraction RP C18 silica gel cartridges have been employed, but the most widely extraction phases employed are polymeric styrene, polystyrene divinil-benzene polymers or N-vinylpyrrolidone. These cartridges are commercially available with different brand names. This solid phase has a medium polarity that allows the selective retention of relatively polar compounds. In this way many different herbicides belonging to various chemical families (sulphonylureas, anilides, imidazolinones, phenoxy acids) have been selectively absorbed and quantitatively analyzed. Solid phase microextraction (SPME) is a very attractive analytical technique for herbicide residue analysis in water. Serôdio (Serôdio et al., 2004) analyzed 13 herbicides which are endocrine disruptors performing a micro extraction using a poliacrilate coated sorptive stirring bar for pesticide extraction, performing a back extraction procedure with acetonitrile to recover all analytes with high selectivity. A miscellaneous procedure for the selective analysis of triazine herbicides in water was presented by (Nelson et al., 2004). A portable system based on immunoextraction and reversed-phase HPLC was developed for the field analysis of herbicides in groundwater and surface water. Using inmunotemplates to concentrate atrazine, simazine, and cyanazine and analyzing them through HPLC, a LOQ of a 0.2mg L^{-1}up to 100mg L^{-1} were achieved. This procedure can be applied to the high throughput analysis of water samples as a single determination took less than 8 min. Another type of class analysis of herbicide residues is the use of molecular imprinted polymers to selectively extract sulphonylureas in water (Bastide et al., 2005). The highly selective polymer concentrates the herbicide residues allowing their determination at the sub ppt level.

MIP technique can be coupled to chemiluminscence sensing, taking advantage of the reaction of many sulphonylureas with luminal+H_2O_2 as reported by (Xie et al., 2010) reaching detection levels of 1-10 nM L^{-1} of sulphonylureas in water. MIP methodology with

its different applications off line, on line or in line has been recently reviewed. Due to the low application doses of herbicides (Table 6) the development of such polymers offers the possibility of sub trace analysis for different herbicide classes. Nevertheless, the improvement of the new LC-MS/MS equipment, allow the detection of many agrochemicals in water, just by filtrating and injecting the sample.

3.1 Herbicide residue analysis in water

In the last decades, liquid-liquid extraction (LLE) has been largely replaced by solid phase extraction (SPE). In 2009 Pinxteren et al., compared the performance of SPE and microwave assisted-solvent extraction (MASE or MAE), both in combination with LC–MS/MS, for the analysis of 10 pesticides in water. The main conclusion of the study was that SPE has the potential of larger sensitivity whereas MASE is faster, provides slightly better recoveries, and represents a promising alternative to conventional off-line SPE concerning low to medium polar compounds (Pinxteren et al., 2009)

One of the advantages of SPE compared to other techniques is the variety of sorbents available. As an example, Geiss et al., compared the performance of 21 cartridges packed with different octadecyl silica, polymeric, modified silica, ion exchange and carbon materials for the extraction of highly polar organophosphorus pesticides from water. In this study the polymeric cartridges were found to have the best performance, and the recovery rates obtained with this material and with the octadecyl solid phases were observed to correlate with the octanol–water partition coefficient of the pesticides (Geiss & Gebert, 2006). Similar results were obtained when testing the extraction of nine herbicides from rice paddy fields water by (Roehrs et al., 2009). Another advantage of SPE is the possibility of automation (Petrovic et al., 2010).

Herbicides in environmental samples such as river, sea or agricultural water are generally highly diluted; therefore a high level of enrichment is required for their detection. As the instruments improve their sensitivity, the pre concentration factor required previous to the analysis is smaller. In reported literature concerning water analysis, as time pass by, the amount of sample required for the analysis is smaller with the clear trend to reduce the sample clean up and pre concentration of the sample.

Ten years ago, Jeannot et al., described two different methods for the analysis of triazines and sulphonylureas in surface waters. They used 0.5-10 L of sample for a liquid-liquid extraction with dichloromethane and two different solid phase extractions (SPE) using the Carbopack B (graphitized carbon black) and the C-18 bonded silica cartridge. The recoveries for each of these methods varied from 83-93% in liquid-liquid mode with RSD between 2-10%, 60-96% in SPE mode on Carbopack B with RSD 3-17% and 67-100% in SPE C-18-bonded silica mode with RSD 2-7% (Jeannot et al., 2000).

In 2003 Zanella et al., validated a method for the determination of 2,4-D, quinclorac, bentazone, clomazone and propanil in surface and agriculture waters. The method was applied for levels between 0.1 and 0.5 mgL^{-1} after a 500-fold-pre-concentration with recoveries ranging from 85.7 to 109.8 % and RSD of 1.8 to 13.4% analyzed by HPLC with UV detection (Zanella et al., 2003). HPLC-DAD was also employed for the determination of these herbicides plus four sulphonylureas, cyhalofop-butyl and bispyribac-sodium but using an OASIS HLB cartridge in tap and rice paddy field waters (Roehrs et al., 2009). The concentration level achieved was 1000 fold detecting herbicides at concentration as low as 40 $ngkg^{-1}$ for tap water and 0.3 μgkg^{-1} for paddy field water.

Ayano et al., in 2004, described a multianalyte method for the determination of five sulphonylureas and three ureas in water. The analysis consisted on a SPE extraction with a polystyrene polymer cartridge (PS2), and ODS C-18-bonded silica cartridge (C-18) and an N-vinylpyrrolidone polymer cartridge (OASIS). The analytes determination and quantitation were performed by liquid chromatography with mass spectrometry (LC-MS). Average recoveries of the eight analytes from water samples were in the range 70-120% with relative standard deviations (RSD) below 20% and the LOQs were between 10 and 100ngL^{-1} (Ayano et al., 2004).

Carabias-Martínez et al., in 2004, developed a method for the simultaneous determination of 10 sulphonylurea and phenylurea herbicides and one of their most common degradation products (3-chloro-4-methyl-phenyl urea) in water. In this procedure LC with diode array UV detection and electrospray mass spectrometry (LC-ESI/MS) in the positive mode were used for the separation, identification and quantification of the selected analytes. A 1000-fold-pre-concentration step based on solid phase extraction was applied for the simultaneous extraction of sulphonylureas and phenylureas from water samples. Three different types of sorbents were compared, silica-based C-18, Oasis HLB and LiChrolut EN obtaining the highest recoveries (70-95%) with the Oasis HLB cartridges (Carabias-Martínez et al., 2004). In 2006, Polati et al., presented the determination of 6 sulphonylureas in surface water. The methodology consisted on a pre concentration/SPE step using a Strata RP-18 E and two polymeric phases, Strata-X and Lichrolut EN, followed by HPLC-UV (240nm)-MS^n analysis. After a 1000/1 pre-concentration the LODs were lower than 26.9ngL^{-1} with recoveries around 81-113% and RSD in the range 10-22 % (Polati et al., 2006).

Kuster et al., in 2007, described the analysis of 14 polar herbicides in the Ebro river delta, during the main growing season of rice, by an automated on-line solid phase extraction followed by liquid chromatography tandem mass spectrometry.

The extraction of the herbicides was performed with polymeric cartridges Hysphere Resin GP (polydivinilbenzene), 10 mL samples were loaded onto the cartridge and the target analytes were eluted directly onto the chromatography column. The detection was performed using a triple quadrupole (QqQ) mass spectrometer and the electrospray interface was operated in both positive and negative mode. Some of the selected herbicides presented difficulties in their recoveries such as alachlor, molinate, propanil, diuron, chlorotoluron, with recoveries below 60% and atrazine, metolachlor, cyanazine with recoveries higher than 120% (Kuster et al., 2007). When analyzing complex matrixes, with minimal work up, matrix effects are noticeable in LC-MS/MS methods and can influence notably the results (identification, quantification and confirmation). The major matrix interferences are due to co-eluting compounds from the sample matrix that can affect the analyte ionization process leading to a signal enhancement or signal suppression. Different approaches have been proposed to overcome matrix effects: sample clean-up, the standard additions method, use of matrix-matched standards, a simple sample dilution if the instrument provides enough sensitivity, but the most widely method employed involves the use of appropriate internal standards. Environmental waters have high sample composition variability and it is difficult to find representative water samples that can be employed for blank determinations and method validation. The problem is still present when using the same type of water (surface, ground) because of their different origin. Therefore, the use of matrix-matched standards, widely applied in analysis of fruits and vegetables, does not provide a straightforward solution in the environmental field. If it is not possible to apply the "dilute and shoot" methodology, the use of analyte isotope-labelled internal standard is

the preferred method to avoid matrix effects influence. This approach has some drawbacks, as only a limited number of reference standards of all possible contaminants are available which are also very expensive. Sometimes other compounds with similar chromatographic properties or structural analogues of the analytes are used but the obtained data is no always good enough (Marín et al., 2009). These authors compared the matrix effects from seven different types of water either using HPLC or UHPLC and the use of labeled internal standards to quantitate 37 pesticides using nine labelled internal standards (I.S). They concluded that in environmental waters, matrix effect was generally a negative effect but no general rules can be applied. Only the labelled pesticide as I.S assured a good correction. Nevertheless for not too loaded waters, the uncorrected values were satisfactory.

In 2009, Mazzella et al. developed a method for the simultaneous determination of 30 triazines, phenylurea and chloroacetanilide herbicides in fresh and estuarine waters, this work addresses two objectives; the development of an accurate method based on ESI-MS/MS detection and the investigation of matrix effects. The pre concentration of the analytes was accomplished by using SPE with Oasis HLB cartridges, with recoveries between 73 to 122% and RSD ranged from 6 to 22% (Mazzella et al., 2009).

Ouyang et al., evaluated the performance of different SPE sorbents for the analysis of 10 sulphonylureas herbicides. The sorbents studied were: silica-based ODS-C18 and two polymeric sorbents, Oasis HLB and Cleanert HXN. Analytes determination and quantitation was carried out with liquid chromatography with electrospray mass spectrometry equipped with ion trap analyzer. The recovery rates range from 76.6 to 109.1% with RSD between 0.3 to 13.8% with the HLB cartridges which was the best one of the three cartridges evaluated (Ouyang et al., 2009).

Also gas chromatography, mainly with MS detection, has been extensively employed for the measurement of herbicide residues in water; different methods had been reported in the literature.

A GC-MS method was developed for the detection of triazine herbicides (atrazine, cyanazine, simazine) and their decomposition products (deethylatrazine, deisopropylatrazine) in environmental waters. The water samples were extracted using an octadecylsilica SPE cartridge with recoveries and RSD in their acceptable ranges (Ma et al., 2003).

SPME combined with GC-MS was developed and employed for the determination of 10 herbicides (alachlor, atrazine, chlorotoluron, diclofop, diflufenicam, ethofumesata, isoproturon, linuron, terbutryn and trifluralin) in surface and ground water. Microextraction was performed with a polyacrylate fiber. It was found that thermally unstable phenylurea herbicides decompose and the resulting anilines can be used for their identification. The recovery of herbicides varied between 94±16 and 107±12% and the detection limit was below 1 $\mu g\ L^{-1}$. It was stated that the method is sensitive, reproducible, easy to perform, and can be applied for the quantitative determination of these herbicides in water (Carabias-Martínez et al., 2003a; Carabias-Martínez et al., 2003b).

In 2008 Crespo-corral et al., describe the determination of carbamate, phenylurea and phenoxy acid herbicide residues by gas chromatography after a potassium ter-butoxide/dimethyl sulphoxide/ethyl iodide derivatization reaction. The method consisted on the pre concentration of the sample using a C-18 sep-Pack cartridge followed by the derivatization of the extract for the analysis with GC-FID and GC/MS in electron impact (EI) and selected ion monitoring mode. The recoveries were in the range 81-99% with RSD 0.9-20.6% (Crespo-Corral et al., 2008).

As a curiosity, phenoxy herbicides have been detected even in rainfall waters in Canada (Hill et al 2002).

3. Herbicide residues analysis in soil

Because of the possible strong binding of herbicides to the polar or apolar components of soil and sediment, the use of an appropriate extraction and pre concentration method is the pre requisite of a reliable chromatographic analysis (Cserháti, 2004). The analysis of pesticides in solid environmental matrices, such as soil, sewage, sludge and sediments, has been addressed in much fewer occasions than in water, probably because of the comparatively greater complexity of the matrices and the absence of environmental quality standards (EQS).

The first and probably the most important step in pesticide residue analysis in soils is soil characterization. There are many reports in the bibliography showing that the efficiency of the extraction method is highly dependent not only on the nature of the soil but also the amount of organic matter in it, being of paramount importance (Merini et al., 2008, Niell et al., 2010). Many nonpolar herbicides are adsorbed to soil organic matter and the cationic herbicides bind tightly to humic acids.

3.3 Sample conservation

In case that the soil sample will not be analyzed immediately, freezing to -20^{o}C is a reasonable measure. Nevertheless, care should be taken if the moist sample is frozen. Sample defrosting takes several hours and during this period microbial growth is exponential and biodegradation of pesticides can occur. Therefore, to prevent the microbial degradation of herbicides it is advisable to freeze-dry the sample and store it properly afterwards. Soil samples without water are easy to handle and to subsample to perform the analysis. To develop the method, the blank soil sample employed has to be treated as the real samples will be handled. Soils can be either reconstructed adding water or extracted directly with the extraction solvent. Nevertheless, it has to be considered that the dryness of the soil increase the clay portion of the soil sorption capacity (Haouari et al., 2006), (Merini et al., 2008), (He et al., 2006) but when water is added the interaction of the herbicide with the humic matter of the soil is increased. These authors also found that the recoveries of 2,4DCP were higher as the water amount of the soil was increased. It is desirable therefore to standardize the water content to the soil by adding a precise amount of water prior of conducting the extraction of the herbicides trapped in the soil.

3.4 Spiking procedure

During the development and validation of the analytical method for soil analysis, the spiking procedure is a crucial step for a successful appropriate analytical method. Pesticides interact in different ways with soils making the reconstruction of that situation in vitro a difficult task. Given that is almost impossible to overcome handicap, there are other points that have to be considered: Spiking solvent selection, volume of the spiking solution, sample homogenization, temperature for solvent evaporation. Merini et al., in 2008, evaluated such parameters for Argentinean loamy soils. They have found that >80% recovery of 2,4 D was obtained using 2000 µl of Methanol as spiking solvent but recoveries were lower using lower volumes. When water was used for spiking, the recoveries dropped down to <50%.

3.5 Extraction solvents

Neutral analytes can be easily extracted using polar organic solvents (MeOH, ACN) or aqueous (MeOH:H_2O, $(CH_3)_2CO$:H_2O) solutions of organic solvents. Ionic herbicides need water solutions to extract them from soils. In many cases, in order to minimize Van der Waals interactions between clay and humic acids, electrolytes like KCl can be employed to

give enough ionic force to the solution to displace the herbicides from the soil. In some cases, the electrolyte also gives an adequate pH to get the most soluble form of the herbicide and to break the interaction with charged soils particles. Particularly sulphonylureas can be easily extracted with MeOH: Aq.0.1M $(NH_4)_2CO_3$ (1:9) from different types of soils having either low or high organic matter content. Nevertheless, the method failed to yield good recoveries from other compounds like cyhalofop-butyl or bentazone. Also ionic compounds like bispyribac sodium cannot be recovered properly (Niell et al., 2010). The most employed herbicides nowadays cannot stand a thermally conducted extraction using methodologies such as MASE or Soxhlet. Pressurized Solvent Extraction and supercritical CO_2 extraction with polar solvents as modifiers are methodologies usually reported for herbicide analysis, but room temperature extractions either ultrasonic or shaker stirred still are the preferred methodologies to perform herbicide extraction from soils. (Baugros et al., 2009; García-Valcarcel & Tadeo, 2009). An example of the influence of the extraction solvents and extraction methodology is shown in the following example. Six chemically different herbicides residues (three sulphonylureas, bentazone, bispyribac-sodium and cyhalofop-butyl) were extracted from four soils of different compositions commonly employed for rice cultivation in Uruguay. The results are shown in Figure 2

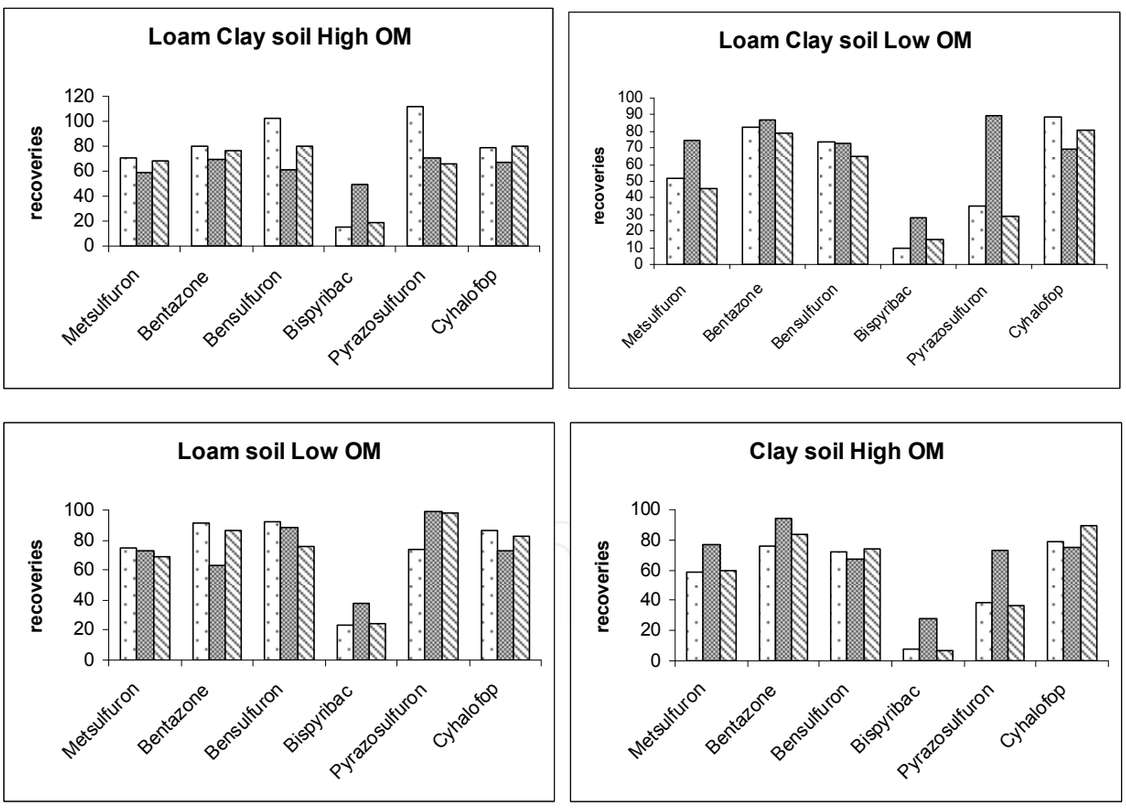

Fig. 2. Recoveries of herbicides from different soil types using two different extraction solvents and two different sitrring procedures (Heinzen et al, unpublished)
- MeOH: AcOEt (7:3),Ultrasonic bath for 15 minutes
- MeOH: 0.1 M $(NH_4)_2CO_3$ (1:9; v/v), magnetic stirred.
- MeOH: AcOEt (7:3), magnetic stirred.

Herbicide recoveries depend on the soil type and the extraction procedure. Different recoveries were observed for the herbicides depending On the type of soil. As a general trend it can be observed that the amount of organic matter usually decreases herbicide recoveries either in clay or loamy soils and the recoveries from loam soils are better than those obtained from clay soils. Aqueous solution of high ionic strength with 10% Methanol gave better recoveries when extracting herbicides from clay soils. Even if the same solvent is used, the stirring method, either ultrasound or magnetic stirring, influences the overall herbicide recovery.

Synthetic herbicides are active at very low doses. Table 6 shows the usual application doses of imidazolinones, sulfonylureas and some individual compounds that are employed worldwide.

Herbicide	kg ha^{-1}	$kgm^{-2}(x10^{-6})$
Sulphonylureas	0.3-0.5	3-5
Imidazolinones	0.1-0.35	1-3.5
Alachlor	0.5-2.0	5-20
Clomazone	1.1	11
Metribuzin	0.57	6
Quinclorac	5-10	50-100

Table 6. Application dose of herbicides per area unit

The concentration of the herbicide applied per m^2 is within the mg range. Taking into account that usually a 1 kg soil sample is made from mixing many subsamples that covered approximately a 10x20 cm of the superficial soil, the amount of herbicide to be detected is in the sub milligram range or ppbs.

In a miniaturized protocol, usually less than 20 g of soil are employed for residue analysis. The detection and quantitation procedure must be sensitive enough to detect the compounds under investigation at such level. The physicochemical properties like the Henry´s law constant and Kow shows that most of the herbicides are either polar or not volatile enough to be GC-analyzed. Nevertheless, in the past, GC/ECD, NPD or sQuad MS operating in the SIM mode detectors were the method of choice to quantify the agrochemicals searched. In many cases, a derivatization step was mandatory as for phenoxy auxin herbicides sometimes indirect detection of the analytes was the only option: thermolabile herbicides like ureas are detected as the corresponding anilines (Berrada et al., 2004). Phenylureas, can be determined directly by GC but some derivatization reactions have been reported to obtain less polar and more volatile compounds. Some of the reagents commonly used are the alkyl iodide and heptafluorobutyric anhydride (HFBA), where the latest one gives very stable derivatization products with a high ECD response. This family of pesticides can also be determined as their thermal decomposition products (isocyanates) that may be obtained quantitatively under closely controlled conditions (Tadeo et al., 2000). Phenylureas also decompose to the corresponding anilines in the GC injection port. This property was applied (Berrada et al., 2004) for the GC determination of phenylureas after MSPE extraction from vegetables with a polyacrilate fiber.

The other old instrumental approach was to use HPLC coupled to UV and fluorescence detectors and later on sQuadMS. The fluorescence detector is very sensitive and selective, and applying pre or post column derivatization, very complicated analytes like glyphosate and AMPA have been routinely analyzed worldwide since twenty years ago.

On the other hand UV detection has many interferences from the soil matrix as it is neither as sensitive nor as specific towards analytes in complex mixtures. Nevertheless, they have been used for years. (Patsias et al., 2002, Niell et al., 2010) Normally, the detection level is Not lower than $0.1 mg kg^{-1} L^{-1}$ in the injected sample. Therefore, sample size must be increased and the extracting solution concentrated in order to get enough analyte to be detected. The main disadvantage is that the other matrix components also concentrate, yielding noisy chromatograms and the herbicide peaks are difficult to identify and in many cases impossible to quantify if a S/N 10:1 has to be reached. HPLC/MS operating in the SIM mode is a more powerful analytical tool and many problems had been solved using this approach, but mainly focused on single residue analysis. The MS detector is also a universal one and interferences from matrices are common, driving to false positives in many cases.

LC-MS/MS techniques are the most powerful methods for pesticide trace analysis particularly herbicides. They offer the possibility of selectively analyze the fragmentation of an ion which was ionized in the first MS cycle. The selectivity achieved and the sensitivity is also remarkable. This advance in the detection and quantitation of trace compounds boosted the strict EU-regulations on contaminant levels allowed in different food matrices. Screening of contaminants is performed mainly with Time of flight instruments (ToF), in MS or MS/MS modes. ToF instruments can determine exact masses for the compounds detected and therefore, unequivocal identification of the analyte is performed. Sorbents used for on-line SPE or MSPE extraction have included both traditional (alkylbonded silica and polymers) (Djozan & Ebrahimi, 2008) and novel molecular imprinted polymers (MIP) materials. MIPs have been proven to be valuable materials for the selective extraction of pesticides (the template molecule and structurally related compounds). The inherent selectivity of the molecular recognition of these materials allows a high degree of sample clean-up to be achieved (Cacho et al., 2009). Zhang et al., reports the preparation of a new non-covalently bonded MIP and its evaluation for pre concentration of metribuzin in soil samples, this MIP was prepared by in-situ polymerization using methacrylic acid (MAA) as the functional monomer and ethylene glycol dimethacrylate (EDMA) as cross-linker. An on-line procedure was, furthermore, employed for the quantitative determination of metribuzin with pre concentration on the monolithic polymer pre-column. MIP can be combined with stir bar extraction for the determination of nicosulfuron (Yang et al., 2010), achieving a sensitivity for water and soils at the nM level. The sample preparation consisted on the maceration of 25 g of soil sample with acetone: water (4:1), followed by filtration and later extraction with dichloromethane in the presence of 20% sodium chloride solution. This sample solution was concentrated and analyzed directly with the on-line procedure. The effectiveness of the MIP was evaluated by LC (Zhang et al., 2009).

In 2008, Lesueur et al., compared four different extraction methods for the analysis of 12 herbicides and two transformation products. The methods were a) ultrasonic solvent extraction (USE) consisting on the extraction of 20g of sample with a 1:2 solution of water/acetonitrile, b) a PLE experiment where 5g of sample were mixed with 1g of silica gel and extracted with acetonitrile/acetone (1:1), c) QuEChERS method and d) European Norm DIN 12393. The analyses were performed by using a LC tandem mass spectrometry system equipped with an electrospray ionization interface operated in positive mode. The lowest recoveries were obtained with the USE method; on the contrary the higher recoveries were obtained with QuEChERS method, between 27-120%. Nevertheless, the lowest limits of detection were obtained with the European Norm DIN 12393 and the highest with QuEChERS and PLE methods (Lesueur et al., 2008).

In 2009, Wu et al., analyzed four sulphonylureas by dispersive solid-phase extraction (dSPE) followed by dispersive liquid-liquid microextraction (DLLME) and HPLC. The dSPE-DLLME procedure consisted on the extraction of 10 g of soil with 20 mL acetone/0.15 M $NaHCO_3$ (2:8) vigorously shaken for 30 min. For dSPE 0.15g of C-18 per 10mL extract was added and shaken. For the DLLME 5mL aliquot of the extract were mixed with 60µL chlorobenzene (as extraction solvent), after vortexing and centrifugation the chlorobenzene residue was dissolved in acetonitrile and analyzed by HPLC. The recovery rates varied from 76-93% with RSD from 5 to 7%. The authors noticed that compared with other conventional sample preparation methods, this analytical technique offers advantages such as simplicity, ease operation, relatively short analysis time, and lower consume of solvent (Wu et al., 2009). Ionic fluids, one of the most popular class of green solvents, are interesting solvents for performing DLLME of herbicides either in water or soil and food aqueous extracts. A room temperature ionic liquid, 1-hexyl-3-methylimidazolium hexafluorophosphate ($[C_6MIM][PF_6]$), was used as extraction solvent and Triton X 114 was used as dispersant. A mixture of 175µL of ($[C_6MIM][PF_6]$) and 50µL 10% Triton X 114 was rapidly injected into the 20mL honey sample by syringe. Herbicides (chlortoluron, prometon, propazine, linuron and prebane) residues were quantified below the 0.01 $mgkg^{-1}$ range (Wang, 2010).
Sulphonylureas have been detected in Canadian wetlands sediment where no agriculture activities had been performed (Degenhart et al., 2010) and in a real situation, a Multirresidue method by Pressurized Liquid Extraction and GC of vineyard soils allowed the simultaneous and GC allowed the simultaneous determination of two herbicides, three fungicides and two Insecticides at ppb level (Schreck et al., 2008).
Diez et al., studied the soil dissipation kinetics of 12 herbicides used on a rain-fed barley crop, the extraction of the soils was carried out with a mixture of acetone, water, and acetic acid (30:7.5:0.3) followed by the analysis in a GC/MS. The extraction method was previously validated for the extraction of 40 herbicides in soils, with recoveries between 71-108% and RSD in the range 0.6-8% (Díez et al., 2008; Díez & Barrado, 2010). The determination by GC-(ITD)MS/MS of triazines, alachlor and metabolites from soils was also performed after Microwave Assisted Extraction (Vryzas et al., 2007).

3.6 Herbicide residues analysis in cereals.

Analysis of herbicide residue involves different steps such as extraction, interference removal, determination of the herbicide residues and their confirmation. All these analysis are generally performed by using several analytical techniques.
The determination of herbicides was initially carried out by colorimetric and spectrophotometric methods but the sensitivity was not enough to meet the regulations and therefore more sensitive methods have been created in order to reach the limits of detection demanded.
The extraction techniques used for the analysis of herbicide residue in cereals depends on the characteristic of the matrix and the polarity of the herbicides.
In recent years, the development of multiresidue methods (MRM) has taken a high profile, however, there are several pesticides that cannot be included in MRM thus single residue methods (SRM) has to be implemented, which means more workload and expenses for the laboratories (Poulsen et al., 2009).
Some examples of herbicides which need these kinds of SRM are glyphosate and their metabolites, acidic herbicides or trazines. Table 7 summarizes different MRM which involve

the determination of herbicides in cereals used during cereals cropping, while in Table 9 the main single extraction methods for some important herbicides are also presented.
Another important factor that the analyst has to take into account is whether the sample suffered an industrial process or if it is raw material. During cereal processing specially during the milling step a big part of the fats components, which are generally found in the bran, are lost. This is an advantage from an analytical point of view, because the sample treatment will not be so exhaustive.

3.7 Extraction and clean-up

Extraction of herbicides from food depends on their polarity and also on the type of matrix. Generally it consists on the homogenization of the sample with an organic solvent alone or mixed with water or pH adjusted, using an ultrasonic bath, a blender or a homogenizer (Lambropoulou & Albanis, 2007).
After the extraction process generally a clean-up procedure is carried out in order to remove the co-extracted compounds that may act as interference during the chromatographic analysis, causing problems in the detection and quantitation of the analytes.
As discussed before, extraction of residues from food depends strongly on the polarity of the selected herbicides, but also on the selectivity and sensitivity of the detection technique employed in their determination.
Nowadays, one of the most used MRM method is QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) but for certain herbicides the use of PSA during the clean-up, or the pH during the extraction causes low recoveries due to poor extraction or chemical interaction with the PSA sorbent. Therefore, several modifications have been implemented in order to improve the recoveries for these kinds of herbicides (CRL website; Anastassiades et al., 2003; Lehotay et al., 2005).
Despite the fact that in routine analysis laboratories prefer the use of few methods with a wide scope, some pesticides, especially some kinds of herbicides require particular extraction conditions and / or clean-up.
The most used herbicides in cereal cropping belong to the phenoxy, phenyl and sulphonylurea families of herbicides. Nevertheless, there are some others like glyphosate, propanil, bentazone (a banned herbicide within the EU) that are also widely used (Herrmann et al., 2006).
This section is focused on the analytical techniques for the determination of most of these herbicides.
The phenoxy herbicides are widely applied as salts or esters but they decompose by hydrolysis, in the plant, to their respective acids thus the extraction of these herbicides is generally carried out with high polarity organic solvents such as MeOH, EtOH or mixtures with water. However these herbicides are best analyzed when they are extracted as their free acids. In general, both acid and basic hydrolysis followed two different strategies. On one hand, samples are hydrolyzed and the free acids extracted with an organic solvent. On the other hand, the parent herbicide and its conjugates are extracted by organic solvents and subsequently hydrolyzed.
Table 7 summarizes the main methodologies used for the analysis of these herbicides in cereals.

Matrix	Extraction Procedure	Clean-up	Ref.	Analytical Technique
Unpolished Rice	Pressurized Liquid Extraction	LLE/SPE:ENVI-Carb/LC-NH_2	(Itoh et al., 2009)	Liquid Chromatography Atmospheric Pressure Photoionization mass spectrometry
Polished rice	QuEChERS (1% HAc in MeCN)	---	(Niell et al., 2010)	Liquid chromatography DAD detection
Barley	QuEChERS	MSPD: PSA, $MgSO_4$	(Díez et al., 2006)	Liquid Chromatography tandem mass spectrometry Gas Chromatography-time of flight-mass spectrometry
Unpolished Rice	Supercritical Fluid Extraction	SPE: BondElut® PSA over Sep-Pak® Florisil column connected in tandem	(Kaihara et al., 2002)	Liquid Chromatography-Electrospray Ionization mass spectrometry
Rice	CH_2Cl_2 ultrasonic bath	SPE: Florisil	(Pengyan et al., 2006)	Gas Chromatography mass spectrometry by selected ion monitoring mode
Cooked polished rice, wheat	MeCN, shaker, ultrasonic bath	---	(Lee et al., 2009)	Liquid Chromatography-Electrospray Ionization mass spectrometry
Rice	QuEChERS	MSPD: PSA, $MgSO_4$/ GPC	(Liu et al., 2007)	Gas Chromatography mass spectrometry
Rice	QuEChERS	MSPD: PSA, MgSO4	(Libin et al., 2006)	Gas Chromatography mass spectrometry
Rice	QuEChERS	Supelclean ENVI-18 cartridge/Supelclean ENVI-carb II/PSA cartridge.	(Takatori et al., 2008)	Liquid Chromatography-Electrospray Ionization mass spectrometry
Rice	QuEChERS	MSPD: PSA, $MgSO_4$, GCB	(Nguyen et al., 2007)	Gas Chromatography mass spectrometry by selected ion monitoring mode
Unpolished rice	Ethylacetate, homogenizer	GPC/ SPE Florisil	(Zhang et al., 2006)	Gas Chromatography mass spectrometry by selected ion monitoring mode
Rice	QuEChERS	MSPD: PSA, $MgSO_4$, GCB	(Nguyen et al., 2008)	Gas Chromatography mass spectrometry by selected ion monitoring mode

Matrix	Extraction Procedure	Clean-up	Ref.	Analytical Technique
Rice, wheat, maize, barley, oats	Accelerated Solvent Extraction	a. ENVI-18 cartridges/ENVIcarb cartridges/Sep-Pak NH_2 cartridge b. Sep-Pak Alumina N cartridge/Sep-Pak NH_2 cartridge	(Pang et al., 2006)	a. Gas Chromatography mass spectrometry, b. Liquid Chromatography-Electrospray Ionization mass spectrometry
Maize	a.Water/1% formic acid in MeCN b. Water/1% formic acid in MeOH c. Water/1% formic acid in acetone	---	(Mol et al., 2008)	Ultraperformance liquid Chromatography electrospay ionization mass spectrometry
Soybean	MeCN:acetone:light petroleum (1:1:1), homogenizer	GPC	(Pizzutti et al., 2007)	Liquid Chromatography-Electrospray Ionization mass spectrometry
Soybean	LLE (MeCN, ethylacetate, 1M phosphate buffer pH 7	SPE: SAX/PSA	(Hirahara et al., 2005)	Gas Chromatography. Detectors FPD, ECD, MSD
Wheat, Rye, Rice, Maize	Buffered QuEChERS	MSPD: PSA, $MgSO_4$	(Herrmann et al., 2006)	Gas Chromatography Ion trap Detection
Wheat	Citrate Buffered QuEChERS	MSPD: PSA, $MgSO_4$, C-18	(Walorckyk, 2007)	Gas Chromatography mass spectrometry
Wheat flour	Water/MeOH, Ultra Turrax	NaCl/ChemElut	(Klein & Alder, 2003)	Liquid Chromatography Electrospray Ionization/atmospheric pressure ionization mass spectrometry
Wheat Flour	Citrate buffered QuEChERS Basic Hydrolysis (optional)	Freezing (optional)	CRL webpage	Liquid Chromatography Electrospray mass spectrometry
Wheat	Citrate Buffered QuEChERS	MSPD: PSA, $MgSO_4$, C-18	(Walorckyk, 2008)	Gas Chromatography mass spectrometry

Table 7. Main methodologies used for the multiresidue analysis of herbicides in cereals.

Herbicides like molinate, atrazine, diuron are normally included and determined in many of the MRM methods listed above along with insecticides and funguicides (Cervera et al., 2010). The following example (Figure 3) shows the multiresidue analysis using HPLC-MS/MS of fifteen herbicides currently employed in rice production. Table 8 summarizes the most relevant analytical features of the analyzed pesticides.

Pesticide	Mode of action	tR (min)	Quantitation MRM1	Confirmation MRM2	Fragmentor (V)	CE1 (eV)	CE 2 (eV)
Azimsulfuron	Sulphonylureas	17.3	447.1/178.1	425.0 / 182.1	120 / 90	10	15
Bensulfuron-methyl	Sulphonylureas	18.7	411.1 / 182.0	411.1 / 149.0	150	20	15
Bispyribac sodium	pyrimidinyloxybenzoic acid	19.2	453.1 / 297.1	453.1 / 179.1	150	15	20
Bromacil	uracyl	14.2	261.0 / 205.0	261.0 / 188.0	90	10	20
Clomazone	unclassified	19.4	240.1 / 125.0	240.1 / 89.0	150	20	60
Cyhalofop-butyl	aryloxyphenoxypropionic	10.4	357.8 / 256.0	357.8 / 302.0	150 / 165	40	12
Fluroxypyr	pyridine	14.5	255.0 / 181.0	255.0 / 209.0	120	20	15
Imazapic	imidazolinone	9.6	276.1 / 163.2	276.1 / 145.0	150	30	40
Imazapyr	imidazolinone	6.5	262.1 / 149.1	262.1 / 217.0	150	30	30
Imazaquin	imidazolinone	14.3	312.1 / 199.0	312.1 / 153.2	90/150	30	50
Imazosulfuron	Sulphonylureas	18.6	413.1 / 156	413.1 / 153.0	150	20	5
Molinate	thiocarbamate	21.4	188.2 / 126.1	188.2 / 55.1	80	10	20
Pyrazosulfuron-ethyl	Sulphonylureas	20.3	415.1 / 182.1	415.1 / 139.1	120 / 90	25	50
Propaquizafop	aryloxyphenoxypropionic	26.3	444.1 / 371.1	444.1 / 100.1	140	15	15
Quinclorac	quinolinecarboxylic acid	14.4	242.0 / 223.0	242.0 / 161.0	90	10	40

Table 8. Instrument acquisition data used for the analysis of the selected herbicides by LC-QqQ/MS tR, retention time, CE, collision energy.

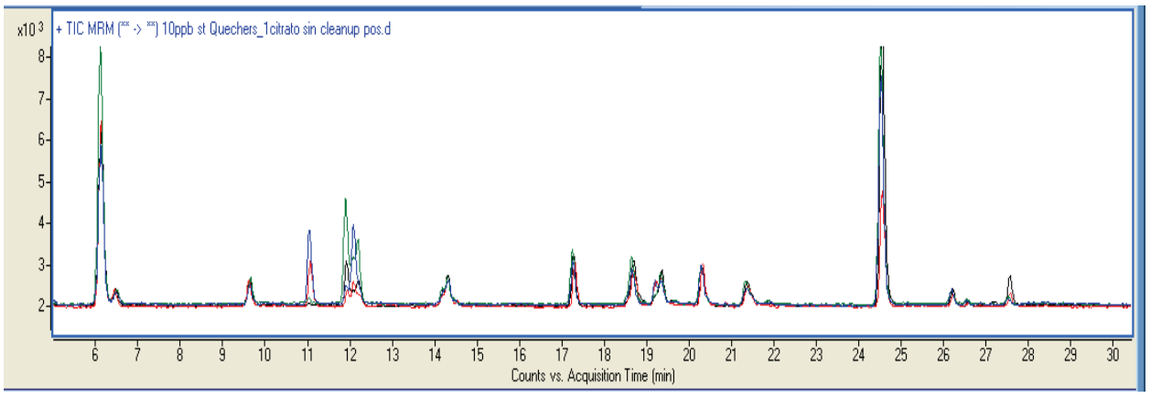

Fig. 3. Analysis of the selected herbicides by LC-QqQ/MS after different QuEChERS procedures at 5µgkg-1 (Pareja et al, unpublished)

For conditions see Table 8.

Different QueChERS based procedures allowed a good extraction and clean up of the complex rice matrix at very low levels, with recoveries ranging between 60-120% (Pareja et al, 2009)

4. Single residue methods

Analyte	Matrix	Extraction Procedure: SRM	%Recovery (% RSD)	Ref.
Glyphosate, chlormequa, mepiquat	Wheat	Glyphosate 3 g of wheat with 25 mL water were extracted twice by ultrasonication. The extract was centrifuged and filtered. The clean up was performed by using a polystyrene reverse phase column. Chlormequat, mepiquat 10 g of sample was extracted with MeOH/water/acetic acid by ultraturrax. The centrifuged extracts were clean-up on a SPE-C-18 column with MeOH/water/ammonium acetate buffer.	89, 93, 90	(Andersen et al., 2007)
Glyphosate	Cereals	1 g of grain flour with 20 mL water were vortexed for 1 min. Overnight standing extraction and then centrifuged for 10 min. Clean up: 1 mL of the extract was loaded into a preconditioned 100 mg C-18-bonded silica cartridge. The cartridge was placed on top of a calibrated tube. 1.5 mL of the supernatant was transferred into the cartridge and by means of over pressure passed through the cartridge and collected into the tube.	88-99 (5.3-7.1)	(Hogendoorn et al., 1999)
Fenoxaprop-p-ethyl, isoxadifen-ethyl	Rice	25 g of sample were extracted with 40 mL acetonitrile/0.1M hydrochloric acid (80:20) and mixed for 10 min on a shaker. The extract was filtered and the paper washed twice with acetone. The filtrate was loaded onto the Chemelut CE250 column and eluted with 150 mL cyclohexane/ethylacetate, the eluate evaporated to dryness.	74.1-98.3 83.3-100.9	(Lucini & Molinari, 2010)
Chlormequat, Mepiquat	Wheat and other cereals flour	3 g of flour were packed into a 11 mL extraction cell and extracted with EtOH at 100 atm at 120 ºC with an extraction time of 15 min divided as follows: preheating (1min), heating (8 min directly set by microprocessor on the grounds of the selected temperature value), static step (5 min) and purging (1 min). Subsequent to extraction 50 μL of IS solution were added to each sample.	83-99	(Marchese et al., 2009)
Triazines	Cereal-based foods	PMAE. 2 g of sample with 20 mL MeOH were placed in the extraction vessel. The magnetron power was set at 100% (600 W). The extraction temperature increased gradually until it reached 105 ºC in that moment the extraction was performed for 10 min. After the completion of the extraction the vessels were allowed to cool at room temperature for 20 min. The extract was filtered and the sediments were rinsed 3 times with MeOH. The mixture was dried by rotatory evaporator at 45 ºC and redissolved in acetonitrile. AMAE. 2 g of sample were refluxed with 40 mL MeOH for 20 min in a modified household microwave oven with 30 % output maximum power of 800 W and a distilling flask fitted with water cooling condenser tube. The extract was filtered and the sediments were	71.9-83.7 (5.3-10.6) 71.5-80.6 (6.9-12.9) 38.9-74.9 (3.8-9.4) 64.5-74.2 (3.3-5.6)	(You et al., 2007)

Analyte	Matrix	Extraction Procedure: SRM	%Recovery (% RSD)	Ref.
		rinsed three times with MeOH. The mixture was dried by rotatory evaporator at 45 ºC and redissolved in acetonitrile. UE 2 g of sample with 40 mL MeOH were placed into a 100 mL flask and extracted in a water bath and sonicated for 90 min. After completing extraction the extract was filtered and the flask rinsed three times with MeOH. The mixture was dried by rotatory evaporator at 45 ºC and reconstituted in acetonitrile. SE 2 g of sample were placed in a glass soxhlet thimble and 40 mL MeOH were added in a 100 mL flask, The flask was fitted with water cooling condenser tube and immersed in a water bath. SE was carried out for 2 h at 100 ºC. After that the extract was filtered and the sediments were rinsed three times with MeOH. The mixture was dried by rotatory evaporator at 45 ºC and rsuspeded in acetonitrile.		
13 phenoxy acids herbicides	Rice	QuEChERS. 10 g of rice with 5 mL water were vortexed for 1 min. 10 ml 0.5 % acetic acid in MeCN were added and shaken two times in vortex. 4 g $MgSO_4$, 1.0 g of tri-Na, 0.5 g di-Na citrate and 1g of NaCl were added, vortexed and centrifuged. Clean up: an aliquot of the extract with 250 mg C-18, 100 mg of alumina neutral and 1.5 g $MgSO_4$, were shaken and centrifuged. 5 mL of the solution were concentrated to dryness and reconstituted for analysis.	45-104 (< 13.3)	(Koesukwiwat et al., 2008)
15 phenylureas herbicides	Rice	Solvent Extraction/SPE. 10 g of rice were homogenized with 50 mL MeCN for 2 min, and filtered into a mixing cylinder and sealed with a stopper, 10 g of NaCl were added and shaked, and the phases were allowed to stand for 20 min. A 25 mL portion of the supernatant was evaporated to dryness, and dissolved in 2 mL n-hexane. Clean up: the extract was transferred to a Florisil SPE column and eluted with 5 mL acetone:n-hexane (40:60), then concentrated to dryness and dissolved in MeCN: water (1:1) for analysis.	75.3-104.3 (1.5-9.6)	(Mou et al., 2008)
Isoproturon	Cereal grains and pasta	Solvent Extraction. 15 g of sample with 4 mL of water and 30 mL acetone were placed in a centrifuged bottle homogenized using an ultra turrax for 30 s at 13500 rpm. Then 30 mL hexane and 30 mL dichloromethane were added to the bottle and homogenized for further 1 min at 13500 rpm. The matrix and extract were separated by centrifugation for 2 min and the extract decanted into a 200 mL volumetric flask. The matrix was resuspended in 30 mL acetone, 30 mL dichloromethane and 30 mL hexane and extracted again for 1 min. The 2nd extract was mixed with the previous one and made up to 200 mL with dichloromethane. A 4 mL aliquot was transferred into a borosilicate glass culture tube, 50 µL of 50%	94-100 (0.8-5.8)	(Winrow et al., 2003)

Analyte	Matrix	Extraction Procedure: SRM	%Recovery (% RSD)	Ref.
		propylene glycol in acetone was added and the mixture evaporated under a nitrogen stream. The residue was reconstituted with 1 mL dichloromethane and placed onto a SPR cartridge containing 200 mg aminopropyl sorbent. The sample tube was rinsed successively with 0.5 mL dichloromethane and 2 mL dichloromethane/MeOH (99:1). The combined eluates were evaporated to nearly dryness and the residue reconstituted in 1 mL MeOH for analysis.		
Phenoxy acids herbicides	Cereals (plant samples)	5 g of sample were extracted with 0.1M NaOH (2x25mL) in a sorvall homogenizer, the extract was filtered and the filter cake washed twice with 5 mL of the basic aqueous solution. The extract was mixed with 25 mL saturated sodium chloride solution and the pH lowered to near 5 by the addition of 2 M H_2SO_4, the solution let stand for 15 min and the liquid decanted. Then the pH of the solution was lowered again to 1 the solution was transferred to a separatory funnel and extracted with diethyl ether (2 x 50 mL). The organic phase was extracted with 0.5 M $NaHCO_3$ (2 x 25 mL), the combined aqueous solution acidified to pH 1 by adding carefully 3M H_2SO_4 (10 mL) and extracted with $CHCl_3$ (2 x 25 mL). The organic phase was filtered through Na_2SO_4 and solvent concentrated to dryness under vacuum.	95.5-104.0 (1.4-10.3)	(Sánchez-Brunete et al., 1994)
Glyphosate, Gluphosinate and their metabolites	Rice, soybean	Rice 5 g of milled rice and 40 mL water were sonicated for 3 min, after resting for 30 min it was centrifuged for the AG1-X8 anion exchange chromatography. Soybean 5 g of soybean sprouts with 25 mL water were mixed in a shaker for 10 min, after resting for 1 min 15 mL acetone were added and centrifuged for the AG1-X8 anion exchange chromatography. 30 mL of soybean extract or rice extract were applied onto a preconditioned AG1-X8/ Dowex 1-X2 column which was first washed with 15 mL 40 % acetone and eluted by repeated elution with 15 mL of 0.5 N HCl five times. Each pooled eluate was dried under reduced pressure at a temperature lower than 55 ^oC for the derivatization reaction and Florisil clean-up. Florisil clean-up of the derivatization products: derivatives in mixed standard solution were dissolved in 1 mL ethylacetate and applied to a Florisil cartridge (500 mg, 6 mL) previously conditioned with 5 mL ethylacetate. The cartridge was eluted with 10 mL ethylacetate followed by 10 mL of acetone, 10 mL acetone / MeOH (1:1) mixture, 10 mL acetone / MeOH (1:2) mixture and 10 mL MeOH. Each pooled fraction of mix standard solution was dried under reduced pressure and then dissolved in 1 mL ethylacetate for analysis.	72-119 (6.7-9.6) 86-101 (4.7-6.5)	(Tseng et al., 2004)

Analyte	Matrix	Extraction Procedure: SRM	%Recovery (% RSD)	Ref.
Trazine herbicides	Wheat	PLE 7 g of sample were mixed with 4.5 g of hydromatrix and placed in a 34 mL extraction cell and extracted with dichloromethane/n-hexane (1:4) solution. The PLE conditions were as follows: pressure 1500 psi, heating time 5 min, purge volume 60%, purge time 100 s, 2 static cycles, static time 6 min. The total extraction time was 21 min, the extract were evaporated to dryness in a rotavapor at 40-50 ^{o}C and the dry residue reconstituted with 5 mL dichloromethane/n-hexane/acetone (1:1:1). The clean-up was performed using OASIS MCX cartridges conditioned with 10 mL dichloromethane, once the retention step has been completed the cartridges were dried under vacuum for 10 min. The elution of the retain compounds was accomplished with 4 mL of 25% ammonia solution /MeOH (15:85). The organic phase was then dried under a stream of nitrogen at 40-50 ^{o}C and reconstituted in 0.5 mL of MeOH/water (1:1) for analysis.	106-125 (6-18)	(Carabias-Martínez et al., 2007)

Table 9. Main methodologies used for the single residue analysis of herbicides in cereals. (Pareja el al unpublished)

Glyphosate is a highly polar herbicide, very soluble in water and insoluble in most organic solvents. For this reason its extraction is generally performed with water or water/chloroform, sometimes at acidic pH, but in this process different components of the matrix are co-extracted thus a clean-up procedure is required (Tadeo et al., 2000).

In Table 9 three different analytical methodologies for the determination of glyphosate in cereals are described. Hogendoorn et al., in 1999 described the rapid determination of glyphosate by means of pre-column derivatization with 9-fluorenylmethyl chloroformate and coupled column liquid chromatography with fluorescence detection. The overall recovery of this herbicide was 86% with a RSD of 9.5%. This procedure implies the extraction with water followed by a clean-up step in a C-18 bonded silica cartridge. The extract was then subjected to derivatization and analysis (Hogendoorn et al., 1999).

In 2004 Tseng et al., reported the analysis of this herbicide and its metabolites in rice and soybean by extraction with water or acetone followed by a clean-up in an AG1-X8/ Dowex 1-X2 anion exchange column and then a single derivatization with trimethylortoacetate in the presence of HAc. The derivative products were then purified using a Florisil cartridge. The detection of these analytes was made by using a gas chromatograph with flame photometric detector. The recoveries and RSDs were 72% and 6.5% for rice and 86% and 6.5 % for soybean respectively with a limit of detection of 20 μgkg^{-1} (Tseng et al., 2004).

In 2007, Andersen et al., organized an intercomparison study for the determination of glyphosate and other herbicides in wheat. The authors described the procedure used by their laboratory for the analysis of these herbicides as follows; the extraction of the analytes with water and then a clean-up step online on a polystyrene based reverse phase column and separated by ion chromatography-HPLC tandem mass spectrometry. The average recovery was 96% with a coefficient of variation of 4% (Andersen et al., 2007).

The EU-CRL for SRM in Stuttgart used a straightforward methanol extraction at pH<2 of wheat flour to validate a new multi class residue method for traditionally analyzed herbicides and metabolites using SRM like glyphosate, AMPA and gluphosinate and MPPA. This procedure was used to analyze some fruits and vegetables matrices.

Glyphosate shares structural similarities with amino acids and therefore has low UV absorption and fluorescence is low and also presents the disadvantage that vaporizes easily upon heating. Thus, it presents difficulties in the quantification by high performance liquid chromatography (LC-UV), LC fluorescence detection and gas chromatography. The analysis of this herbicide by gas chromatography requires its derivatization, this procedure involves the use of trifluoroacetic anhydride (TFAA) and trifluoroethanol, TFAA and diazomethane or HBFA and 2-choroethanol (Tadeo et al., 2000).

Gas and liquid chromatography with classical detectors.

Gas chromatography is widely used in the analysis of herbicide residues, due to the high selectivity and sensitivity of the detection systems.

The classical detectors most often used are the flame ionization detector (FID), nitrogen-phosphorus detector (NPD), Thermo ionic detection, electro capture detection (ECD), but in the last decade MS coupled to GC has been the choice for the analysis of herbicides, especially in MRM methods (Tadeo et al., 2000).

The NPD is employed for herbicides containing nitrogen, such as triazines, dinitroanilines or chloroacetamides, this detection system allows limits of detection in the range of μg-mg kg^{-1}.

In the case of ECD, this detector has high sensitivity for halogenated compounds, although its linear range is narrow. It has been used in the analysis of halogenated phenoxyacids, benzonitriles, dinitroanilines, glyphosate and multiresidue analysis, frequently after derivatization.

Liquid Chromatography (LC) is very useful for polar, thermally labile and low-volatility pesticides which in general cannot be directly analyzed by GC, so LC is preferred instead of the use of derivatization techniques.

Most pesticides, including those not easily analyzed by GC, can also be separated by high-performance liquid chromatography without the need of chemical derivatization. phenylureas, organophosphorus pesticides, triazines, quaternary ammonium compounds and chlorinated phenoxy acids are examples of pesticides submitted to LC analysis (Thurman et al., 2005).

Conventional LC detectors such as the UV detector are, however, not selective enough for pesticide analysis in complex matrices. Moreover, selective detectors such as fluorescence detection can only be applied after derivatization.

GC-MS/MS and LC-MS/MS

Over the last 20 years liquid chromatography–mass spectrometry (LC–MS) techniques have advanced dramatically in their sensitivity, specificity and reliability. Detection of sub-ppt concentrations is becoming routine for many organic analytes and methods achieving detection of a few hundred femtograms of some analytes have been reported. Such progress Is mostly due to the development of hyphenated LC–tandem MS techniques, which are today the methods of choice for the determination of trace organic analytes in food and environmental samples. Such growth in the use of LC–MS/MS for the analysis of organic contaminants in environmental matrices has been compelled by the need for high-quality

data on their occurrence in the environment at very low concentration levels (Petrovic et al., 2010).

5. Conclusion

Herbicides are integrated to the general cropping systems. Routine herbicide application in intensive crop production is performed following a more or less strict calendar but the number of applications can be more frequent if a weed suddenly threatens the culture. The rotational no-tillage based productive system winter crop-summer crop uses many herbicides like atrazine, sulphonylureas and imidazolinones that can accumulate in soils and in a carry-over phenomenon injure the newly planted crop. Herbicides can be found in every environmental compartment but their occurrence in water, soils and crops are of paramount importance from a toxicological point of view. Herbicides that have low Kow and Koc, can be found in water, either ground or surface through run off, leaching or lixiviation. Therefore herbicides can be found not only in the application site as they migrate through water flows. On the other hand, high Koc and /or Kow herbicides remain trapped in soils. Many different analytical methodologies have been developed to determine pesticide residue concentrations. Herbicide residue analysis is not only a valuable tool to evaluate threshold damage limits for the carry over phenomenon but also their residual levels knowledge are fundamental to evaluate the sustainability of the global agroecosystem. The development of chromatographic hyphenated MS/MS techniques allow the broadening of MRMs scope and therefore, lower detections limits with high accuracy have been reached for a high number of pesticides, but the most commonly developed MRM include only few herbicides. The challenges of herbicide residue analysis are the low application dose of the active substances and therefore, sample handling and clean-up procedures are still the bottle neck of the analytical methodologies. Selective clean up procedures have been developed to isolate and concentrate the searched compounds during the last years. In this context, an increasing amount of methods for different food and environmental matrices have been published. New analytical procedures based on selective polymer extraction (MIP, SPME) are interesting possibilities. As general procedure, SPE extraction is the method of choice for water. Nevertheless, for herbicide residue analysis in soil, the type of soil determined the scope and clean up methodology to follow. After the success of the GMO soybean RR glyphosate-resistant variety, the trend for new crop varieties introduction is based on their selective resistance to specific herbicides like imidazolinones or gluphosinate and sulphonylureas. If these herbicides will be applied in no-tillage production systems at the same ratio as glyphosate is nowadays, environmental issues will probably arise, as imidazolinones and sulphonylureas are semi persistent molecules.

6. References

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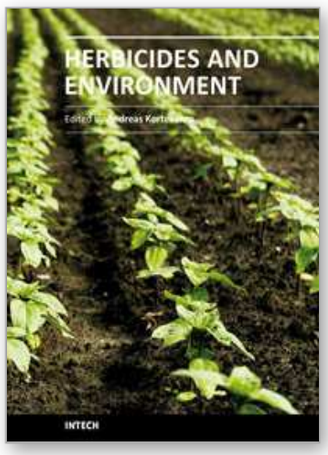

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Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

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