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Evaluation by HPLC-UV of Polar Pesticides in Rice Fields

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Carbamate pesticides have become very popular in the last few years because of their wide spectrum of biological activity. Analytical methods for determining carbamate pesticides have recently been reviewed (Liska and Slobodnik 1996; Barceló and Hennion 1995). Widespread use of these pesticides can lead to water pollution. In Europe a list of pesticides used in amounts of over 50 tons per year and their capacity as probable transient leachers was published (Fielding et al. 1992).

The Natural Park of La Albufera, located at the South of the city of Valencia (Spain), forms a part of a group of townships and occupies a total of 21119 ha and a water receiver basin of 2837 ha. The humid parts consist of the lake, irrigation channels and rice fields. This protected area has been considered a Special Protection Zone for birds (Directive EEC/409/79). However, contamination by pesticides has been observed in several studies (Picó et al. 1994, Ferrando et al. 1992). Of the pesticides applied to the land around the Albufera Lake, molinate, thiobencarb and carbofuran are three of the most widely used. The amount of pesticide applied to rice fluctuates from year to year and depends on the weather, acreage planted and availability. Most of the herbicides and insecticides are applied to flooded fields only once, usually in May and June when the plants are small. Physical properties as water solubility, K_{OC} K_{OW} and half life ($t_{½}$), indicate, in a simplified manner, the tendency of a determined pesticide to degrade or remain in water, i.e. a pesticide with K_{oc}<l00 and $t_{½}$ <l00 days has an important leaching power. In contrast, a K_{oc}>100 and a long $t_{½}$ mean a greater possibility to be carried by water.

Molinate and thiobencarb are used in large amounts, because of its physical properties (see Table l), molinate has a certain tendency to leach depending on the nature of the soil, but its residues normally remain in water, and can be degraded microbially. As the water solubility of thiobencarb is much lower than that of molinate and its K_{OC} is much higher, it is much more likely to be adsorbed by soil and thus avoid leaching processes. Its main degradation pathway is also microbial.

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Table 1. Physical properties of the two pesticides found in La Albufera Lake.

	Molinate	Thiobencarb
$t_{1/2}$ (days)	14 / 35	14 / 21
K_{oc}	186	1380
K_{ow}	223	2630
Water solubility (mg/L, 20°C)	800 / 912	30
Stability	Stable: -to hydrolysis -1 month at 120°C -2 years at room t^e -1 month at pH: 5, 7 and 9 at 40°C	Stable: -in acid media -under sunlight -1 month at pH 5-9 at 21°C
Application rate (kg/ha)	55-65	40-50

In this study twelve sites from La Albufera were sampled from April 1997 to September 1998 to determine the presence of twelve pesticides using a Solid-Phase Extraction (SPE) method and HPLC-UV previously studied and optimized.

MATERIALS AND METHODS

Samples were taken once a month between April 1997 and September 1998, at twelve sites along the irrigation channels for rice fields in La Albufera lake (Valencia, Spain). The sites were: Gola Pucol, Racó de L'Olla, Tancat de Malta, Acequia Obera, Acequia Dreta, Acequia Rodena, Acequia Canal, Acequia Llosa, Acequia Riuet, Acequia Cuarenta Palmos, Gola Perelló and Gola Perellonet. Samples (4 L) were collected in amber glass bottles filled to the brim, and stored immediately after sampling always at 4°C until analysis. No preservation procedure was used and storage time was minimal (one week or less).

Pesticide standards (purity >98%); carbaryl, carbendazim, carbofuran, dietofencarb, dioxacarb, fenothiocarb, iprodione, methomyl, methylthiofanate, molinate, oxamyl and thiobencarb were from Promochem. Solvents (Merck) were for pesticide residue analysis. Stock solutions (1 and 0.2 µg/L) of individual pesticide standards were prepared in acetonitrile. A mixed stock solution (1.5 µg/L) containing all twelve standards was prepared from stock solutions of individual pesticides by mixing and diluting with acetonitrile.

The analytical procedure has been described previously (Jiménez et al. 1995). Basically, it consists of a previous filtration of samples through a Whatman No. 1 filter, addition of 10% of NaCl, and an extraction (250 mL) using 0.5 g of C8. Elution from solid phase was done with 5 mL acetonitrile followed by 5 mL dichloromethane. The eluate was air dried and made up to 0.2 mL in acetonitrile, and finally 20 µL of the concentrate was analyzed with the HPLC system using a water/acetonitrile gradient and detected at 210 nm.

RESULTS AND DISCUSSION

The limits of detection (LODs), calculated using HPLC-grade water, were under the European Union limit for drinking waters of 0.1 µg/L for carbaryl, carbofuran, dietofencarb and iprodione. Carbendazim, dioxacarb, fenothiocarb, methylthiofanate, molinate and thiobencarb were slightly over this limit. LODs obtained were 0.16, 0.11, 0.18, 0.22, 0.18 and 0.21 µg/L, respectively. Pesticide behaviour in natural media is affected by many factors such as temperature, pH, organic matter or surfactants. The pH can vary, especially at the surface level, as a result of agricultural practices. The use of fertilizers, for example, can produce an acidification when ammonia nitrogen is used, and the organic matter content contributes substantially to the absorption of the pesticide, affecting the bioactivity, bioaccumulation, biodegradability, leachability and volatility of these products. In general, high levels of organic matter absorbe pesticides, thus favouring transformation and degradation processes and consequently, inhibiting their transport to underground waters. As temperature, pH, organic matter and surfactants may affect pesticide extraction, these three parameters were determined in all the samples to determine any possible relationship between them and the efficacy of pesticide extraction.

Temperatures, determined in the sampling site, ranged from 1.5 to 23°C. The pH of the water samples was measured, and no variations were found; the pH ranged from 6.17 to 8.08. Determinations of oxidability to potassium permanganate, an indicator of the organic matter present in waters were achieved. The complete method follows the APHA Guidelines (APHA, 1986). The method used (421 C) was applicable to the natural waters studied due to their low concentration of organic matter. The suitability of the method was confirmed by comparing the results obtained with those achieved with a Chemical Oxygen Demand (COD) kit consisting of a potassium dichromate oxidation solution and spectrophotometric quantification. Analysis demonstrated that no extreme values were to be found in the water samples. After sample filtration, the levels of organic matter were so low (between 2.06 and 7.65 mg O_2/L) that they did not affect pesticide extraction. The oxidability levels were high in non-filtered waters probably because of anthropogenic pollution of the waters by industrial and municipal waste. Some irrigation channels are used to wash away residues that should be sent to depuration plants.

Surfactants were determined following the relative simple and precise APHA method 512 B (APHA, 1986) for anionic surfactants calculated as methylene blue active substances (MBAS). It comprises three consecutive extractions from acid aqueous medium containing an excess of methylene blue into chloroform, followed by an aqueous backwash and measurement of the blue colour in the chloroform by spectrophotometry at 652 nm. The minimum detectable quantity is about 10 µg MBAS calculated as Linear Alkylbenzene Sulfonate (LAS). This method has been applied successfully to the examination of the anionic surfactant content in drinking water supplies. The natural waters collected in this study were analyzed after a filtration that lead them to very clean waters, thus they could be considered as drinking waters, in the terms of absence of interference for the cited APHA method. The influence that some surfactants have on the SPE of the tested pesticides was examined in an earlier study (Jiménez et al 1995) and, on the whole it can be deduced that levels assayed did not interfere. Nevertheless, the presence of surfactants was determined in order to confirm that their levels were too low to affect pesticide extraction. Surfactants were practically absent in all the samples. These results indicate that no interference from pH, organic matter and/or surfactants affected the pesticide extraction in the analyzed samples.

As a first step SPE of the samples was used for the preconcentration, and with the method used an enrichment factor of 1250 fold was afforded, and allowed the liquid chromatographic analysis of ten of the twelve selected pesticides. The two more polar pesticides, oxamyl and methomyl, were eluted very early under our tested conditions due to the slight retention by the Cl8 column and the fast elution under defined gradient conditions. A larger proportion of water at the beginning of the gradient program just lead to a union of subsequent peaks, but not to a proper determination of methomyl and oxamyl. These two peaks, spiked to natural waters coincide with co-extracted natural water impurities. The chromatograms of a blank, a spiked to 1.2 µg/L for each pesticide, and a positive natural water extracted are presented in Figure 1. The pesticides analyzed at the one-month intervals from April 1997 till September 1998 were detected only in five months, comprised between May 1997 and June 1998. The results including positive water are given in Table 2. The most usual compounds are two herbicides, molinate and thiobencarb. Molinate appears in 46 of the 216 samples, followed by thiobencarb, which appears in 40 samples. The maximum concentration of pesticides (2182 µg/L) was found in May 1997 for the whole group. In May 1998 the pesticide levels were also the highest of the year, but the top amount found for the totality of the irrigation channels was lower than in 1997; 154.9 and 35.4 µg/L for molinate and thiobencarb in 1998, while in 1997 the values were 1608.6 and 573.4 µg/L respectively. These levels present a decreasing tendency in the two months after the highest concentration, and all the intakes were negatives from August 1997 till May 1998; the reductions ranged from 55.6 % to 100 %. The concentrations of molinate were, in all the samples higher than those of thiobencarb and ranged from 0.2 to 1383 µg/L for a concrete irrigation channel. Other studies demonstrate that carbamate pesticides have been found in many rivers and lakes around the world (Pereira and Hostletter 1993, Picó et al. 1994, Kobayashi et al. 1993, Chiron et al. 1995, Tanabe et al. 1996, Gruber and Munn 1998). The rate of dissipation for molinate is very rapid as was demonstrated previously (Deuel et al. 1978) and its half-life varies from 96 to 54 hours depending on the type of application: intermittent irrigation or continuous-flow irrigation. Therefore, the high levels found in the analyzed waters mean that the sample intake occurred very few days after the application to the rice and that the levels of pesticides used were high.

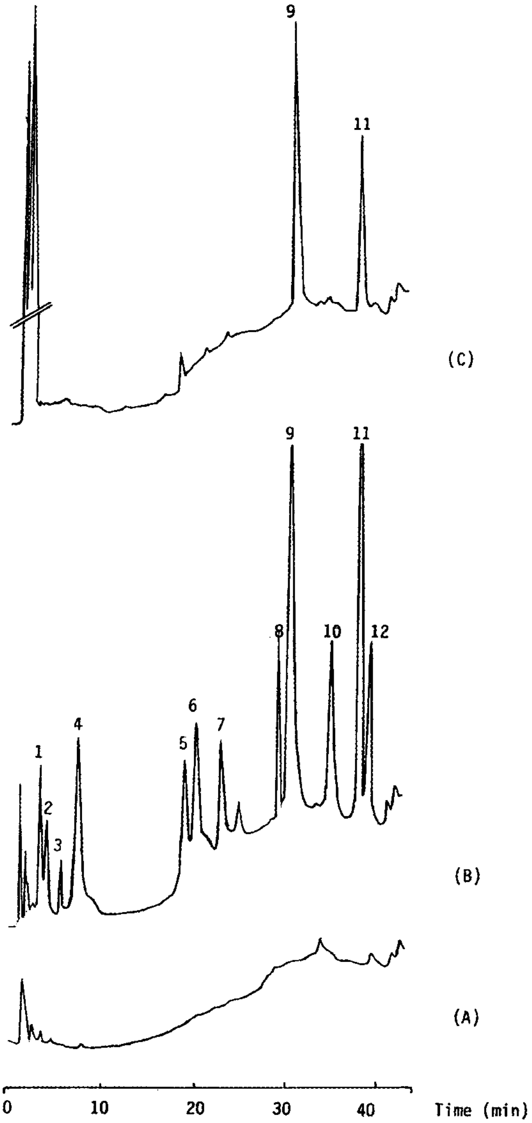

Figure 1. Chromatograms after injecting (A) blank extract of deionized water, (B) standard mixture and (C) a positive natural water from Racó L'Olla (1997) all of them under the conditions described in the text. 1.- oxamyl, 2.- methomyl, 3.- dioxacarb, 4.- cadazim, 5.- methylthiofanate, 6.- carbofuran, 7.- carbaryl, 8.- dietofencarb, 9.- molinate, 10.- fenothiocarb, 11.- iprodione, 12.- thiobencarb.

Table 2. Levels of pesticides (µg/L) in the positive samples from the La Albufera lake and irrigation channels from May 1997 to September 1998.

SAMPLING SITE	PESTICIDE	SAMPLING DATE				
		May '97	June '97	July '97	May '98	June '98
Gola Puçol	molinate	4.5	1.6	2	nd	nd
	thiobencarb	nd	nd	1.1	nd	nd
Racó L'Olla	molinate	0.9	nd	0.5	nd	nd
	thiobencarb	0.58	nd	0.3	nd	nd
Tancat Malta	molinate	24.4	5.8	0.3	2.3	0.9
	thiobencarb	5.2	2	0.2	1.5	0.3
Acequia Obera	molinate	16.8	6.7	nd	2	2.5
	thiobencarb	9.1	3	nd	2	0.2
Acequia Dreta	molinate	1.8	nd	0.2	16.2	3
	thiobencarb	1	nd	0.1	3.2	0.3
Acequia Rodena	molinate	25.8	4.3	2	54.7	3.7
	thiobencarb	18.6	1.7	1	10.2	nd
Acequia Canal	molinate	26.9	10.3	nd	8.4	2
	thiobencarb	5.3	3.6	nd	8.1	nd
Acequia Llosa	molinate	52	23.4	3	16.1	nd
	thiobencarb	11.9	4.8	0.5	1.6	nd
Acequia Riuet	molinate	16.2	7.2	nd	18.7	2.9
	thiobencarb	7.4	2.4	nd	3.5	nd
Acequia 40 Palmos	molinate	12.4	6.7	nd	8.9	nd
	thiobencarb	2	4.4	nd	0.8	nd
Gola Perelló	molinate	1383	4.9	nd	29.6	5.3
	thiobencarb	505	1	nd	4.5	0.5
Gola Perellonet	molinate	43.6	12	nd	10.8	4.2
	thiobencarb	7.9	2.6	nd	3.4	0.3

nd: not detected

The thiobencarb levels were lower than those of molinate, ranging from 1.6 to 573 µg/L for a concrete irrigation channel. Thiobencarb dissipates fairly rapidly upon field application, and a field dissipation half-life of 5-7 days has been reported (Crosby 1983) although this always depends on the type of application (soluble, powder ...). Iwakuma et al. (1993) detected thiobencarb in waters at levels of 10 ppb, which is much lower than those found in La Albufera lake and surroundings. The irrigation channel with the highest content in herbicides is the Gola Perello, located near the water outlet from the lake to the sea, with a total concentration during the monitoring period of 1934 µg/L, followed by the Acequia Rodena with 122 µg/L of both pesticides.

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