Environ. Sci. Technol. **1998**, *32,* 2180−2184

Evaluation of ELISA Kits Followed by Liquid Chromatography-Atmospheric Pressure Chemical Ionization-Mass Spectrometry for the Determination of Organic Pollutants in Industrial Effluents

M. CASTILLO, A. OUBIÑA, AND
D. BARCELÓ*
Department of Environmental Chemistry, CID-CSIC,
c/Jordi Girona 18-26, 08034 Barcelona, Spain

Contaminated industrial effluents often contain a variety of organic pollutants which are difficult to analyze by standard GC−MS methods since they often miss the more polar or nonvolatile of these organic compounds. The identification of highly polar analytes by chemical or rapid biological techniques is needed for characterization of the effluents. The present work will evaluate the use of enzyme linked immunosorbent assays (ELISA) kits for determining pentachlorophenol, carcinogenic PAHs and BTEX (benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylene) among the organic analytes present in various industrial effluents from Europe (petrochemical plant and hazardous waste). The analytical protocol applied for the evaluation of the kits was based on the use of ELISA followed by solid-phase extraction (SPE) for the preconcentration of a variety of organic pollutants such as pentachlorophenol, phthalates, and nonylphenol and final determination with LC−MS characterization using an atmospheric pressure chemical ionization (APCI) interface in the positive and negative ionization modes. The developed protocol permitted the unequivocal identification of target analytes such as pentachlorophenol, nonylphenol, dibutylphthalate, dimethylphthalate, bis(2-ethylhexyl)phthalate, 2-methylbenzenesulfonamide, and 2,2-dimethylbenzenesulfonamide present in industrial effluents. The advantages and limitations of the three RaPID-magnetic particle-based ELISA kits applied to the characterization of industrial effluents are also reported.

Introduction

A large number of organic chemicals has been discharged into the environment as a consequence of industrial activities. Many of them represent a hazard for the environment due to their toxicity and/or accumulation in sediments and organisms. As a consequence, strict characterization of contaminated effluents needs to be done. In this respect, the European Union (EU) has promulgated several years ago the so-called “black list” of 132 dangerous substances (Directive 76/464/EC) of target analytes that should be monitored as dangerous substances discharged into the aquatic environment (*1*). In the last year a new Directive on Integrated Pollution Prevention Control (IPPC) has been promulgated by the European Union (Directive 96/61/EC) expanding the range of pollutants that should be monitored in industrial effluents discharges such as those from paper and pulp industry, refineries, textiles, and many other sectors (*2*). In this perspective, research in the area of characterizing new pollutants in contaminated industrial effluents will be encouraged and will be expanded during the next coming years.

Common methods for identifying organic pollutants in contaminated industrial effluents involve generally the use of either dichloromethane liquid−liquid extraction (LLE) or solid-phase extraction (SPE) followed by gas chromatography−mass spectrometry (GC−MS) techniques (*3*−*8*). By GC−MS a variety of nonpolar compounds are generally determined but all the polar, ionic, heavy, and thermally unstable compounds comprising more than 95% of the total organic carbon (TOC) content (*9*) cannot be analyzed. In this case, LC techniques are a good alternative as less sample cleanup is required, thermally labile compounds are more easily analyzed, derivatization is usually not required, and polar and high molecular weight compounds can be identified.

LC has been rarely reported in the characterization of polar analytes detected in industrial effluents. The U.S. EPA has published two methods for the analysis of solid waste (SW-846) involving either particle beam (U.S. EPA Method 8325) or thermospray (U.S. EPA Method 8321) (*10*, *11*). Nowadays, the development of atmospheric pressure ionization (API) LC−MS interfaces allows one to obtain structural information similar to chemical ionization techniques overcoming the limitations of other LC−MS interfacing devices such as poor structural information or sensitivity such as for TSP and PB, respectively. In a previous paper (*12*), we have developed an off-line SPE method followed by LC-atmospheric pressure chemical ionization-MS for the characterization of organic pollutants in industrial effluents.

Environmental monitoring generally requires analyzing a large number of samples; therefore, there is a need to search low cost, rapid, and automated methods for analysis. In the past few years, one of the most developed field-testing methods has been enzyme linked immunosorbent assays (ELISA) (*13*, *14*), which have acquired a wide acceptance within the U.S. especially. In this respect, the U.S. EPA has recently released the official method 4010A for the determination of pentachlorophenol (EPA method 4010A) (*15*) among other methods for PCBs, PAHs, TNT, and pesticides. Previous work from our group (*16*) has evaluated the use of the RaPID PCP kit for the determination of PCP in certified wastewaters and soil samples.

In the present work, three RaPID-magnetic particle-based ELISA kits for determining pentachlorophenol, carcinogenic PAHs, and BTEX (benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylene) were applied to the characterization of industrial effluents. The final objective is to evaluate the three commercially available kits for the determination of target pollutants present in contaminated industrial effluents in order to comply with the recently introduced directive. Nontarget compounds will be evaluated as interferences in the ELISA kits. All the industrial effluents evaluated by ELISA will be afterward analyzed by solid-phase extraction (SPE) followed by LC-atmospheric pressure chemical ionization (APCI)-MS. In this way, a complete characterization of the organic pollutants present in the effluents will be achieved.

---

* Author to whom correspondence should be addressed. Fax: 34-3-2045904; e-mail: dbcqam@cid.csic.es.

S0013-936X(97)01042-0 CCC: $15.00

Experimental Section

Chemicals and Reagents. LC-grade solvents and pentachlorophenol were obtained from Merck (Darmstadt, Germany). Ethylbenzoate and phthalates were from Aldrich (Milwaukee, WI). Tributyl phosphate was obtained from Kodak (Rochester, NY). 4-Nonylphenol was from Kao Corporation (Barcelona, Spain). The rest of the standards were a gift from Mario Negri Institute (Milano, Italy). Acetic acid pro-analyze grade from Panreac (Barcelona, Spain) was used. The RaPID-magnetic particle-based ELISA assays for pentachlorophenol, carcinogenic PAHs, and BTEX from Ohmicron (Newtown, PA) were purchased through Merck (Barcelona, Spain). PBST was 0.2 M phosphate buffer with 0.8% saline solution (pH 7.5) containing 0.05% Tween.

Sample Collection. Samples from industrial effluents were collected in Pyrex borosilicate glass containers. Each bottle was rinsed with tap water and with high-purity water prior to sample addition. Sample preservation was accomplished by storing the bottles at 4 °C immediately after sampling. Treated plant effluent was collected at the discharge pipe (effluent) of a petrochemical plant during July 1996 (sample A_1), January 1997 (sample A_2), and March 1997 (sample A_3). Additional sampling was also conducted during December 1996 (sample B_1) and March 1997 (sample B_2) at an industrial landfill leachate (sample B). More information about sample B was reported by Benfenati et al. (*3*) using other GC−MS procedures.

Sample Preparation. Off-line SPE experiments were performed using an automated sample preparation with extraction columns system (ASPEC XL) fitted with an external 306 LC pump for the dispensing of samples through the SPE cartridges and with an 817 switching valve for the selection of samples from Gilson (Villiers-le-Bel, France). The details of the preconcentrating step have been described in a previous paper (*12, 17, 18*). The styrene-divinylbenzene sorbent Lichrolut EN (200 mg, 6 mL) from Merck was used for off-line SPE purposes. We have indicated that after preconcentration the sorbent was completely dried to avoid hydrolysis of the trapped compounds. The drying step was carried out using a Baker Spe 12G apparatus from J. T. Baker (Deventer, Netherlands). The fact of using a drying step was needed for the phenolic type and more polar analytes; however, we have observed losses for the more volatile PAHs (see Table 1). This could only be avoided if no drying step took place, but then the other analytes studied suffered important losses.

LC-APCI-MS Conditions. For LC-APCI-MS experiments, a VG Platform from Micromass (Manchester, U.K.) equipped with a standard atmospheric pressure ionization (API) source which can be configured for APCI or ISP was used. The APCI interface consists of a heated nebulizer probe and a standard atmospheric pressure source equipped with a corona discharge needle. The solvent was delivered by a Waters 616 gradient pump system controlled by a Waters 600 S controller from Waters-Millipore. The operation conditions were optimized in a previous work (*12*). Source and probe temperatures were set at 150 and 450 °C, respectively, corona discharge voltage was maintained at 3 kV, and the cone voltage was between 20 and 40 V. The HV lens voltage was set at 0.20 kV. In full scan mode, the *m*/*z* range was from 80 to 400 in both negative ion (NI) mode and positive ion (PI) modes of ionization.

As total of 100 μL of the extracts was injected in the LC system using as mobile phase water and acetonitrile, both acidified with 0.5% of acetic acid following the next gradient: from 30% of acetonitrile and 70% of water in isocratic conditions during 15 min to 100% of acetonitrile in 15 min and back to initial conditions in 5 min at a flow rate of 1 mL/min. A Hypersyl Green ENV column (150 mm × 4.6 mm i.d.) equipped with a guard column both from Shandon *HPLC* (Cheshire, U.K.) was used.

TABLE 1. Mean Recoveries and (in parentheses) RSD% (n = 3) Obtained in Loading Different Volumes of Industrial Wastewater Spiked at 50 μg/L in the Target Analytes Using Off-Line SPE with Lichrolut EN (*12*, *18*)

compd	V_{load} = 300 mL	V_{load} = 500 mL	V_{load} = 900 mL
fluorene	43 (8)	30 (12)	19 (12)
phenanthrene	65 (7)	46 (9)	34 (10)
anthracene	62 (6)	47 (8)	37 (10)
fluoranthene	102 (7)	89 (7)	79 (7)
benzo[*a*]pyrene	105 (6)	101 (7)	83 (6)
indeno(1,2,3-c,d)pyrene	109 (6)	94 (6)	88(7)
pyrene	106 (6)	90 (7)	80 (7)
catechol	55 (13)	<5	<5
phenol	57 (17)	<5	<5
acridine	55 (15)	<5	<5
4-methylphenol	63 (11)	24 (10)	<5
2,4-dinitrophenol	59 (11)	32 (15)	23 (13)
2,2′-biphenol	88 (10)	66 (11)	43 (12)
4-nitrophenol	79 (13)	64 (12)	48 (13)
3,3′-dichlorobenzidine	108 (9)	104 (9)	51 (12)
2-nitrophenol	89 (11)	97 (12)	71 (13)
naphthol	97 (8)	107 (9)	69 (9)
benzidine	86 (9)	95 (8)	77 (9)
1-methylindol	92 (8)	97 (9)	95 (10)
benzophenone	105 (11)	104 (9)	103 (10)
3,3′−dimethylbenzidine	103 (9)	99 (10)	104 (10)
dibutylphthalate	54 (7)	48 (10)	41 (9)
dimethylphthalate	69 (15)	55 (13)	46 (9)
4-nonylphenol	34 (5)	26 (6)	13 (9)
pentachlorophenol	87 (9)	85 (8)	79 (11)
2-methylbenzenesulfonamide	22 (8)	9 (7)	<5
2,2-dimethyl-1,3-propanediol	24 (9)	11 (9)	<5
bis(2-ethylhexyl)phthalate	63 (9)	58 (10)	44 (9)

Determination of most of the organic compounds present in the effluents was carried out using the previously reported MS data (*12*). For PAH analysis, the *m*/*z* ions reported by Anacleto et al. were used (*19*).

Immunoassay Procedure. The samples were assayed according to the RaPID Assay package insert described by Oubiña et al. for pentachlorophenol determinations (*16*). The protocol for carcinogenic PAHs and BTEX assays was very similar to the pentachlorophenol assay; the main difference was the first incubation time of 20 and 15 min, respectively, instead of 30 min. The spectrophotometric measurements were determined using the RPA-I RaPID Photometric Analyzer (Ohmicron, Newtown, PA). Detailed operations of RaPID ELISA kits have been previously described by our group (*13, 14, 16*).

Results and Discussion

Recoveries and Breakthrough Volumes. A preliminary study of SPE previous to the analysis of the samples was performed. In this sense, the above-mentioned off-line SPE method was applied for the preconcentration of wastewater samples spiked with 50 μg/L of different analytes. Target compounds were chosen according to the origin of the samples and considering a compendium of contaminants commonly found in chemical disposal sites (*3, 4, 9, 16, 17*). Loading volumes of 300, 500, and 900 mL were preconcentrated (in triplicates) in the Lichrolut EN cartridges to evaluate the recoveries and breakthrough volumes of target compounds. Table 1 shows the main recoveries and relative standard deviation (RSD%) obtained for the target compounds in the preconcentration of different sample volumes using off-line SPE with Lichrolut EN. Part of the data reported was obtained in previous experiments (*12, 18*).

TABLE 2. Identified Compounds by Off-Line SPE LC-APCI-MS and Their Estimated LOD and Concentration (Results Corrected from Recovery) Obtained in the Monitoring of Wastewaters from a Petrochemical Plant in July 1996 (Sample A_1), January 1997 (Sample A_2), and March 1997 (Sample A_3) and of an Industrial Landfill Leachate during December 1996 (Sample B_1) and March 1997 (Sample B_2)

identified compounds	concn (μg/L)					LOD (μg/L)
	sample A_1	sample A_2	sample A_3	sample B_1	sample B_2	
ethylbenzoate	51.0	ND	ND	54.5	ND	3.8
4-nonylphenol	12.0	ND	ND	ND	ND	2.9
pentachlorophenol	0.4	ND	ND	ND	ND	0.3
isothiocyanate-cyclohexane	11.2	ND	ND	ND	ND	0.9
1,1,3,3-tetramethyl-2-thiourea[a]	39.3	100.8	34.5	ND	ND	1.9
bis(2-ethylhexyl)phthalate[a]	0.1	30.0	10.5	3.0	ND	0.1
1-methyl-2-pyrrolidinone[a]	ND	66.3	33.7	0.2	3.9	0.2
diisooctylphthalate[a]	ND	70.5	26.9	ND	40.5	0.1
tributyl phosphate	ND	ND	ND	0.66	ND	0.2
dibutylphthalate	ND	ND	ND	0.78	ND	0.1
benzylbutylphthalate	ND	ND	ND	ND	3.0	0.1
2-methylbenzenesulfonamide	ND	ND	ND	1.88	ND	0.2
dimethylphthalate	0.6	ND	ND	ND	ND	0.1
2,2-dimethyl-1,3-propanediol	ND	ND	ND	14.7	ND	1.0

[a] Periodically found compounds; ND not detected.

Good recovery values (from 79 to 109%) were obtained in the preconcentration of 300−900 mL for the most nonpolar compounds (i.e., some PAHs), although the most polar analytes such as 2-methylbenzenesulfonamide and 2,2-dimethyl-1,3-propanediol were not detected when preconcetrating 900 mL due to breakthrough. When preconcentrating 300 mL, recoveries for certain polar analytes such as acridine, dibutylphthalate, 4-nonylphenol, 2-methylbenzenesulfonamide, and 2,2,-dimethyl-1,3-propanediol were 55, 54, 34, 22, and 24%, respectively. These results indicated that the detection of these polar compounds was only feasible by loading volumes of 300 mL of water at most. There are other compounds such as fluorene, catechol, and phenol, with recovery values of 43, 55, and 47%, respectively, when loading volumes of 300 mL of water. In this specific case, it is known that these analytes are quite volatile and so losses in the drying step after the preconcentration of the water samples occur. When this drying step is not incorporated, then recoveries reached 75%, although in that case, recoveries of many analytes decrease dramatically.

Another aspect to be considered when examining the recoveries for the PAHs fluorene, phenanthrene, and anthracene is that it looks like the breakthrough volume of these analytes occurs at values below 300 mL of water, but this is not the case. These compounds, due to their chemical structure, exhibit much higher breakthrough volumes than 900 mL of water, but important losses in the drying step, during sample preparation occur, although a careful manipulation of the samples took place. In addition to that, slight adsorption onto the particles and vessels can occur, so lowering as well their recoveries (*18*).

In general, we should indicate that the industrial wastewater matrix has a negative effect on the recoveries. In this respect, total organic carbon (TOC) was also measured in samples A_1, A_2, and A_3 with values of 13, 25, and 33 mgC/L which are higher as compared to surface waters used for drinking purposes (4−7 mgC/L) but are much lower than those found in highly contaminated industrial wastes (*9*). Therefore, it is important to consider the influence of the water sample on efficiency of the SPE process. The wastewater matrix is characterized by the presence of interferences and particles that can decrease the effectiveness of the SPE process by plugging the pores of the sorbent. This was noticed for phenolic compounds. When using industrial wastewater instead of groundwater, the recoveries decreased by 25% (*12*). So, as compromise, the protocol described in the Experimental Section was used for all the target analytes, from nonpolar to polar analytes present in industrial wastewaters.

LC-APCI-MS. Characterization of water samples was carried out by means of LC−MS techniques. This approach allows us to identify and to obtain information for the most polar, heavy, and thermally unstable pollutants which cannot be analyzed using GC techniques. An atmospheric pressure chemical ionization interface was used, and its efficiency to detect target compounds was checked before analyzing wastewater samples (*12*). It is interesting to note that $[M + H]^+$ and $[M - H]^-$ ions were detected in PI and NI modes, respectively, for almost all target compounds (except isothiocyanate-cyclohexane in PI mode and 4-nonylphenol in PI and NI mode). This technique allows us to obtain important molecular weight information.

The developed method of SPE followed by LC-APCI-MS was applied to the analysis of industrial effluents (A and B). Both NI and PI modes with extraction voltages set at 20 and 40 V were used for real samples in order to detect as many compounds as possible. All compounds were identified by matching retention time and MS spectrum in PI and NI modes with authentic standards.

The chromatographic profile obtained in full scan mode showed chromatographic peaks containing unresolved components. Nevertheless, SIM chromatograms provided a more selective method avoiding the presence of interferences. Table 2 lists all the compounds observed in samples from sites A and B and their estimated concentrations and limits of detection (LOD) for the developed off-line SPE coupled to LC-APCI-MS method.

Pentachlorophenol (PCP) was identified, it is a priority analyte frequently formed in bleaching processes, and its presence in industrial effluents has been reported (*16*). 4-Nonylphenol was also confirmed in sample A_1 by injecting an authentic standard. Nonylphenols are degradation products of nonylphenol ethoxylates (nonionic surfactants) often added to separators to break up oil−water emulsions. They have also been used as plastic additives. Recently, they were found to be estrogenic (*20*) apart from being persistent, lipophilic, and toxic to aquatic organisms with a 96-h LC_{50} values for salmon and trout ranging from 0.13 to 0.23 mg/L (*21*). Phthalates were repetitively found in the petrochemical plant wastewaters. Their concentration level ranged between 70.5 μg/L for diisooctylphthalate down to 0.6 μg/L for dimethylphthalate. These phthalates supposedly originated from plastics. Although they are not acute toxic compounds, their presence in the environment should be controlled due

TABLE 3. Responses (%) and Their Coefficients of Variation (in Percentage, $n = 3$) Obtained with Water Spiked at the Same Concentration Found in the Wastewater Samples Using the Pentachlorophenol and the Carcinogenic PAHs RaPID Assay

	% recovery	
compds	pentachlorophenol RaPID assay	carcinogenic PAHs RaPID assay
tributyl phosphate	0.11 (5)	37 (2.6)
dibutylphthalate		31 (6.2)
dimethylphthalate		42 (10)
ethylbenzoate		0.16 (8.9)
4-nonylphenol	0.75 (2.9)	2 (11)
pentachlorophenol	100 (1)	
1-methyl-2-pyrrolidinone	47 (10)	147 (16)
2-methylbenzenesulfonamide	77 (12)	124 (5.8)
1,1,3,3-tetramethyl-2-thiourea	0.3 (1.6)	0.68 (3.0)
isothiocyanate-cyclohexane		1.45 (6.9)
2,2-dimethyl-1,3-propanediol		0.93 (2.3)

to their persistence in the water medium and to their hormone disrupting effects (*22*). 1,1,3,3-Tetramethyl-2-thiourea was detected in January 1997 (sample A_2) at of 100 μg/L. This analyte is listed on the U.S. EPA list of toxic substances. Thiourea compounds are mainly used as accelerators in the rubber industry, and they are frequently found in PVC plastic or adhesive, diazo paper, and paints or glue remover (*23*). The rest of pollutants are present in lower concentrations. Nevertheless, all of them (except ethylbenzoate) are included in the lists regulated by the U.S. EPA, OSHA, or EC, and therefore, its control is needed to prevent alarming levels.

ELISA Determinations. The analytical interference due to the limited selectivity of antibodies, commonly referred to as cross-reactivity (CR), is one of the most important aspects to take into account to correctly estimate the final values of the ELISA determinations (*24*). To characterize the selectivity of the antibodies, a battery of cross-reactants (structurally similar compounds that may compete for the antibody-binding sites) had to be checked. In our case, the selected analytes were obtained from the data reported in Table 2, since all these analytes were unequivocally identified and quantified by SPE followed by LC-APCI-MS. Two phthalates (dibutylphthalate and dimethylphthalate), a non-ionic detergent metabolite, 4-nonylphenol, tributyl phosphate, ethylbenzoate, 1-methyl-2-pyrrolidinone, 1,1,3,3-tetramethyl-2-thiourea, isothiocyanate-cyclohexane, 2,2′-dimethyl-1,3-propanediol, and 2-methylbenzenesulfonamide were tested in the pentachlorophenol and carcinogenic PAHs tests, to be used to detect the possible increase of the final concentration of the target analytes.

Table 3 shows the mean responses using the two tests obtained with groundwater spiked at the same concentration level found in the wastewater samples of Table 2. Data of Table 3 was obtained by spiking the water samples with individual compounds and then applying the appropriate ELISA kit. The most remarkable data correspond to the carcinogenic PAHs kit, because all the studied compounds could be detected, whereas the pentachlorophenol kit only responded to six of them (pentachlorophenol, ethylbenzoate, 4-nonylphenol, 1-methyl-2-pyrrolidinone, 2-methylbenzenesulfonamide, and 1,1,3,3-tetramethyl-2-thiourea).

Table 4 lists the values obtained in samples from sites A and B using the pentachlorophenol, carcinogenic PAHs, and BTEX ELISA tests. The BTEX test detects petroleum hydrocarbons (benzene, toluene, ethylbenzene, and *m*-, *o*-, and *p*-xylene) expressed as the sum of the six components. The total BTEX RaPID assay presents a sensitivity, Least detectable dose (LDD) based on a 90% B/B_0, of 0.02 mg/L. The carcinogenic PAHs RaPID assay detects carcinogenic PAHs and related compounds to different degrees referred in the CR data of the kit. So the possible cross-reactants can increase this final concentration. The sensitivity of this assay (as benzo[*a*]anthracene) in water is 0.01 μg/L. Pentachlorophenol ELISA test can also detect a variety of related compounds, especially chlorophenols as it was shown in a CR study using this kit with a sensitivity of 0.03 μg/L (*16*).

TABLE 4. Concentrations of Pentachlorophenol, Carcinogenic PAHs, and Petroleum Hydrocarbons (Commonly Referred to as BTEX) Found in Four Industrial Wastewaters Using the Pentachlorophenol, Carcinogenic PAHs, and BTEX RaPID ELISA Kits[a]

	ELISA PCP (μg/L)	ELISA PAHs (μg/L)	ELISA BTEX (mg/L)
sample A_1	0.32 (3)	1.88 (8)	nd
sample A_2	0.45 (10)	1.99 (6)	nd
sample A_3	7.01 (9)	2.82 (3)	nd
sample B_1	4.43 (2)	7.8 (8)	15.3 (8)

[a] Total BTEX, equivalent parts of benzene, toluene, ethylbenzene and *m*-, *o*-, and *p*-xylene, is expressed as the sum of the six components. A_1, A_2, and A_3 are wastewater samples of a petrochemical plant collected during July 1996, January 1997, and March 1997, respectively, and B_1 is a wastewater sample from an industrial leachate collected in December 96. Coefficient of variation is in parentheses (in percent, $n = 6$). nd, values below detection limit.

The wastewater samples could be analyzed directly by immunoassay without previous filtration or cleanup. BTEX could only be detected in samples B_1. This is not surprising since the sensitivity of the BTEX kit is approximately 3 orders of magnitude lower that the other two ELISA kits. Since the concentrations of the different analytes that will be present in the industrial wastewater and that can give CR with the test are in micrograms per liter, the BTEX ELISA kit was not studied for CR, as for the other ELISA kits (see data of Table 3). The high value obtained in sample B_1 cannot certainly be attributed to the compounds listed in Table 2 since the BTEX ELISA is reported in milligrams per liter, whereas the LC−MS data of Table 2 are in micrograms per liter. This ELISA kit should deserve another study, but from our experience, taking into consideration the levels found in most of the industrial wastewaters, the BTEX ELISA kits offers poor sensitivity for the trace determination of organic pollutants in such type of samples.

The data reported in Table 4 indicates also that all samples gave a positive answer with the carcinogenic PAHs and PCP kits. Considering the data reported in Table 3, the values of Table 4 correspond, not only to the target analytes, but also to the cross-reactants present in the wastewater samples. So, in this way, ELISA determinations can be used as a measurement of the related contamination present in the wastewater samples. In this respect, and taking into account the relatively high values of carcinogenic PAHs obtained by ELISA (see Table 4) and as no PAHs were detected in those samples (see Table 2), these values can be attributed in a first instance to phthalates that exhibit high CR (see Table 3). For a better estimation of the PAH kit, blank wastewater samples without any PAHs and related analytes that can give cross reactivity will need to be determined. This experiment was not conducted because it would be difficult “a priori” to know which analytes do not cross-react with the PAHs kit.

The good correlation between chromatographic and ELISA determinations could be noticed in sample A_1. Pentachlorophenol was detected in this sample by LC-APCI-MS at 0.4 μg/L, whereas 0.32 μg/L were obtained by ELISA. In this case, although the pentachlorophenol should be detected at 100%, the presence of other cross-reactants, 4-nonylphenol and 1,1,3,3-tetramethyl-2-thiourea, can interfere the detection of the target analyte, but in practice has low influence. As reported earlier (*24*), the percentage of the CR can vary versus the cross-reactant concentrations, showing low values at higher doses of these cross-reactants. For samples A_2, A_3, and B_1, the PCP ELISA kit give response with 0.45, 7 and 4 μg/L, respectively. Since no PCP was present in the sample, this should be related to cross-reacting analytes, such as 2-methylbenzesulfonamide and 1-methyl-2-pyrrolidinone, that exhibit high values of CR with this ELISA kit (see Table 3). Other phenolic compounds that were either not detected or could not be unequivocally confirmed could also be the responsible for the PCP ELISA values, as we have observed in a previous work when analyzing wastewater samples (*16*).

Another important point to take into account is the absence of previous treatments such as filtration or cleanup, showing that these results can only be used as a screening tool of wastewater samples and for giving a rapid estimation of the contamination of these wastewater effluents.

In summary, three different kits, pentachlorophenol, carcinogenic PAHs, and BTEX RaPID assay have been used for screening purposes of four wastewater samples. Even though overestimation could be observed due to the absence of treatments in the ELISA determinations and the interferences due to the cross-reactants detected by LC-APCI-MS, the combination of both techniques, automated systems such as ASPEC XL for SPE followed by LC-MS and immunoassays, open a new window for monitoring organic compounds present in industrial effluents.

Acknowledgments

This work has been supported by the project entitled “Protocol for the Evaluation of Residues in Industrial Contaminated Liquid Effluents (Pericles)” (Contract ENV4-CT95-0021) from the Environment and Climate Program, 1994−1998, Commission of the European Communities and CICYT (AMB96-1675-CE). We thank Merck for supplying the SPE cartridges. S. Galassi, E. Benfenati, and F. Ventura are thanked for providing toxicological fractionated industrial waste samples, standards, and for carrying out the TOC measurements, respectively.

Literature Cited

(1) Directive 76/464/EC, OJ No L 129 of May 18, 1976.
(2) Directive 96/61/EC, OJ No L 257 of October 10, 1996.
(3) Benfenati, E.; Facchini, G.; Pierucci, P.; Fanelli, R. *Trends Anal. Chem.* **1996**, *15*, 305−310.
(4) Betowski, L. D.; Webb, H. M.; Sauter, A. D. *Biol. Mass Spectrom.* **1983**, *10*, 369−376.
(5) Burkhard, L. P.; Durhan, E. J.; Lukasewycz, M. T. *Anal. Chem.* **1991**, *63*, 277−283.
(6) Hale, R. C.; Smith, C. L. *Int. J. Environ. Anal. Chem.* **1996**, *64*, 21−33.
(7) Ellington, J. J.; Thurston, R. V.; Sukyte, J.; Kvietkus, K. *Trends Anal. Chem.* **1996**, *15*, 215−224.
(8) Barceló, D.; Porte, C.; Cid, J.; Albaigés, J. *Int. J. Environ. Anal. Chem.* **1990**, *38*, 199−209.
(9) Betowski, L. D.; Kendall, D. S.; Pace, C. M.; Donnelly, J. R. *Environ. Sci. Technol.* **1996**, *30*, 3558−3564.
(10) U.S. EPA Method 8321A. Solvent extractable non volatile compounds by high performance liquid chromatography/particle beam/mass spectrometry (HPLC/PB/MS) or ultraviolet (UV) detection. U.S. EPA, Office of Solid Waste and Emergency response: Washington, DC, 1995, pp 1−50.
(11) U.S. EPA Method 8325. Solvent extractable non volatile compounds by high performance liquid chromatography/thermospray/mass spectrometry (HPLC/TS/MS) or ultraviolet (UV) detection. U.S. EPA, Office of Solid Waste and Emergency response: Washington, DC, 1995, pp 1−50.
(12) Castillo, M.; Alpendurada, M. F.; Barceló, D. *J. Mass Spectrom.* **1997**, *32*, 1100−1110.
(13) Gascón, J.; Durand, G.; Barceló, D. *Environ. Sci. Technol.* **1995**, *29*, 1551−1556.
(14) Oubiña, A.; Ferrer, I.; Gascón, J.; Barceló, D. *Environ. Sci. Technol.* **1996**, *30*, 3551−3557.
(15) U.S. EPA Method 4010A. Screening for Pentachlorophenol by Immunoassay. U.S. EPA: Washington, DC, January 1995; pp 1−17.
(16) Oubiña, A.; Puig, D.; Gascón, J.; Barceló, D. *Anal. Chim. Acta* **1997**, *346*, 49−59.
(17) Castillo, M.; Puig, D.; Barceló, D. *J. Chromatogr., A* **1997**, *778*, 301−311.
(18) Pérez, S.; Ferrer, I.; Hennion, M. C.; Barceló, D. *Anal. Chem.* (Submitted for publication).
(19) Anacleto, J. F.; Ramaley, L.; Benoit, F. M.; Boyd, R. K.; Quilliam, M. A. *Anal. Chem.* **1995**, *67*, 4145−4154.
(20) Castillo, M.; Barceló, D. *Trends Anal. Chem.* **1997**, *16*, 574−583.
(21) Lee, H. B.; Peart, T. E. *Anal. Chem.* **1995**, *67*, 1976.
(22) Jobling, S.; Reynolds, T.; White, R.; Parker, M. G.; Sumpter, J. P. *Environ. Health Perspect.* **1995**, *103*, 582−587.
(23) Raffaelli, A.; Pucci, S.; Lazzaroni, R.; Salvadori, P. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 259−264.
(24) Oubiña, A.; Gascón, J.; Barceló, D. *Anal. Chim. Acta* **1997**, *347*, 121−130.

Received for review December 4, 1997. Revised manuscript received April 9, 1998. Accepted April 27, 1998.

ES971042Z