Bull. Environ. Contam. Toxicol. (1994) 52:25–30

Environmental Contamination and Toxicology

Determination of Pesticide Residues in Natural Waters of Greece by Solid Phase Extraction and Gas Chromatography

George Emm. Miliadis

Pesticide Residues Laboratory, Benaki Phytopathological Institute, 7 Ekalis Str., GR-145 61 Kifissia, Greece

Environmental contamination of natural waters by pesticide residues is of great concern. Pesticides are divided into many classes, of which the most important are organochlorine and organophosphorus compounds. Organochlorine pesticides are known to resist biodegradation and therefore they can be recycled through food chains and produce a significant magnification of the original concentration at the end of the chain. Organophosphorus pesticides on the other hand are known to degrade rapidly depending on the their formulation, method of application, climate and the growing stage of the plant (Khan 1980). Pesticide residues reach the aquatic environment through direct run-off, leaching, careless disposal of empty containers, equipment washings etc.

The analysis of ultratrace levels of pesticide residues in water is fraught with many technical difficulties. Quantitation at the 0.1-µg/L level, that is required by the E.C. Council Direcive (1980) for water intended for human consumption, requires very careful steps of analysis, in order to avoid false positive identifications and low accuracy of the measurements.

In the present study a simple and fast solid phase extraction technique was employed for the extraction, and two different gas chromatographic procedures were used for the identification and quantitation of pesticide residues, mainly organophosphorous and organochlorine, in natural waters of Greece. To date, very little work has been done on monitoring pesticides in the natural waters of Greece (Albanis et al. 1986, Miliadis 1992).

MATERIALS AND METHODS

Water samples were collected in 2.5 - L glass bottles, between October 1992 and December 1992, from the following lakes : Amvrakia, Himaditida, Iliki, Lysimahia, Marathon, Meg. Prespa, Mic. Prespa, Mornos, Petron, Plastira, Trihonida, Vegoritida and Zazari, and the following rivers : Aliakmon, Arahthos, Drosopigi, Enipeas, Gortsilakas, Lailia, Louros and Pinios. Many of these lakes and rivers provide Athens and other big cities in Greece with potable water. Samples were stored at 4°C prior to extraction, which was normally conducted within 24 hr of sampling.

The solvents used were "pesticide residue" grade. Stock solutions (1000 and 100 µg/mL) and working solutions (10, 1, 0.1 and 0.01 µg/mL) of lindane, aldrin, dieldrin, phorate, parathion ethyl and parathion methyl, as well as solutions of mixtures of these pesticides were prepared in acetone according to the standard practices (Chau and Lee 1982).

Solid phase extraction (SPE) using pre-packed reversed-phase octadecyl (C 18)-bonded silica, contained in cartridges, was used for sample preparation. Sep-Pak Plus cartridges (Millipore Corporation) containing 400 mg of packing material were preconditioned by passing six cartridge hold-up volumes (5 mL) of acetonitrile with a glass syringe. This was followed by 5 mL of methanol, which was subsequently displaced with 10 mL of organic-free water. 1-L of water sample was cleaned by removal of any floating or insoluble material. A glass tube, connected through a teflon tubing with the cartridge, was inserted into the water sample and the other end of the cartridge was joined to vacuum chamber. Suction was adjusted to ca 800 mbar in order to obtain a flow rate of 15 mL water/min. through the cartridge. After passing the sample, suction continued for 10 min. to dry the packing material. The cartridge was disconnected, and the absorbed pesticides were eluted by passing ethyl acetate through the cartridge, with a glass syringe, into a 5 - mL volumetric cylinder. Ethyl acetate was added until bringing extract in the cylinder to 2 mL above any water. The extract was then transferred in a sealed tube for gas chromatographic analysis.

A Varian aerograph model 3700 gas chromatograph was used with two detector systems a) a Ni-63 electron capture detector (ECD) with a 2m X 2mm i.d. glass column containing 1 : 1 mixture of 10% OV-101 and 15% OV-210 on Chromosorb WHP (80 - 100 mesh) and b) a nitrogen - phosphorus detector (NPD) with a 2m X 2mm i.d. glass column containing 10% OV-101 on Chromosorb WAW (80-100 mesh). Operating temperatures were : injection port 220°C, column oven 200°C, detector 300°C (ECD) and 250°C (NPD). Nitrogen carrier-gas flow rate was 60 mL/min. 1µL of the sample extract was injected for each chromatographic system and two chromatograms were obtained for each sample. Quantification was achieved by use of a computer integrator.

RESULTS AND DISCUSSION

Six target pesticides were selected in this study to assess the applicability of the method. Three of them are widely used organophosphorous pesticides (phorate, parathion methyl and parathion ethyl) and the other three typical organochlorine (lindane, aldrin and dieldrin).

Application of the SPE technique to the determination of pesticide residues in water is currently gaining acceptance, after been validated by different official institutions (Junk and Richard 1988). Acetonitrile was used to elute the concentrated pesticide residues, as it was found to be a superior eluting solvent compared to methanol and acetone.

The ECD and NPD chromatographic separation and detection systems were selected so that, identification of unknown peaks could be performed by comparison of the relative retention time (RRT) of the unknown peaks to the RRTs given in various RRT data tables (U.S. Deptm. of Health 1982, tables 331-B and 333-A). The RRTs of the six target pesticides were found to coincide with those expected from the data tables.

For statistically evaluating the extraction efficiency of the 6 target pesticides by the SPE technique, 1 L of high purity water was fortified with the compounds at various concentration levels. Figure 1b shows gas chromatogram of a fortified water sample. The results of the recovery study are presented in Table 1. The average recoveries were between 73 and 104% except for aldrin with 42-49%, and relative standard deviations between 4 and 14%, values satisfactory for residue analysis (Greve 1984).

Quantitation of compounds in samples was made by comparing the detector response for the sample to that measured for the calibration standard, within the linear range. Using standard mixtures within the ranges of concentration shown in Table 1 the response was found linear, with correlation coefficients $r \geq 0.998$ for all analytes except for parathion methyl with $r = 0.992$ and parathion methyl with $r = 0.984$.

A conservative estimate of the method detection limit is the product of the worst case standard deviation at the lowest validation level with the Student t-value (U.S., E.P.A. 1984), which is 6.96 at 99% confidence level for 2 degrees of freedom (3 replicates). The detection limits evaluated by this approach are : 0.004 μg/L for lindane and aldrin, 0.01 μg/L for dieldrin, 0.03 μg/L for phorate and 0.06 μg/L for parathion methyl and parathion ethyl.

Comparing the SPE procedure with the ethylene chloride extraction method, used in an earlier study (Miliadis

Table 1. Mean recovery (%) ± relative standard deviation (N=3) for target pesticide residues in fortified water samples, at various fortification levels (μg/L). Li = lindane, Al = aldrin, Di = dieldrin, Ph = phorate, PM = parathion methyl, PE = parathion ethyl.

μg/L	Li	Al	Di	Ph	PM	PE
0.01	99± 6	48± 5				
0.02	102± 4	42± 4	79±10			
0.04			78± 8			
0.05	101± 6	44± 5				
0.1	99±10	47± 8	85± 9	83± 4		
0.2	99±10	49±12	75± 9	91± 4	101± 4	102± 4
0.4			91±11		99± 5	98± 9
0.5				77± 8		
1				73± 5	101± 5	99± 6
2				87±11	99±14	92± 6
4					104± 5	95± 4

1993), it is found that SPE is more economical, requires fewer operational steps, while providing acceptable reproducibity and analyte recoveries. The recovery data of the two procedures were compared by Student's t-test. There were no significant differences (P=0.05) between recoveries of the two methods for dieldrin, parathion methyl and parathion ethyl. However, SPE recoveries were significantly higher for lindane and phorate, and significantly lower for aldrin. Standard deviations of the two methods were compared with the F-test and were found not to differ significantly at the 5% significance level. Limits of detection were also found similar for the two methods.

Thirty three water samples collected from the mentioned rivers and lakes of Greece were analyzed using the SPE procedure. A method blank (1 L of high purity water) was analyzed with each set of samples. This was necessary since small interfering peaks arose, especially with the electron capture detection. These peaks have been attributed to phthalate plasticizers in the polypropylene housing of the cartridges (Junk et al, 1988). No pesticide residues were detected in any water samples, except those from Iliki lake. Lindane was detected in these samples at concentration of 0.015 μg/L. Figure 1a shows a chromatogram with the ECD for Iliki lake water. Confirmation of lindane was achieved by the 10% OV-101 column with the ECD. Lindane (gamma-hexachlorocyclohexane) is an insecticide which is applied to the soil of potato fields lying adjacent to Iliki lake. However concentration of lindane found in water is much lower than the EEC maximum acceptable concentration of 0.1 μg/L.

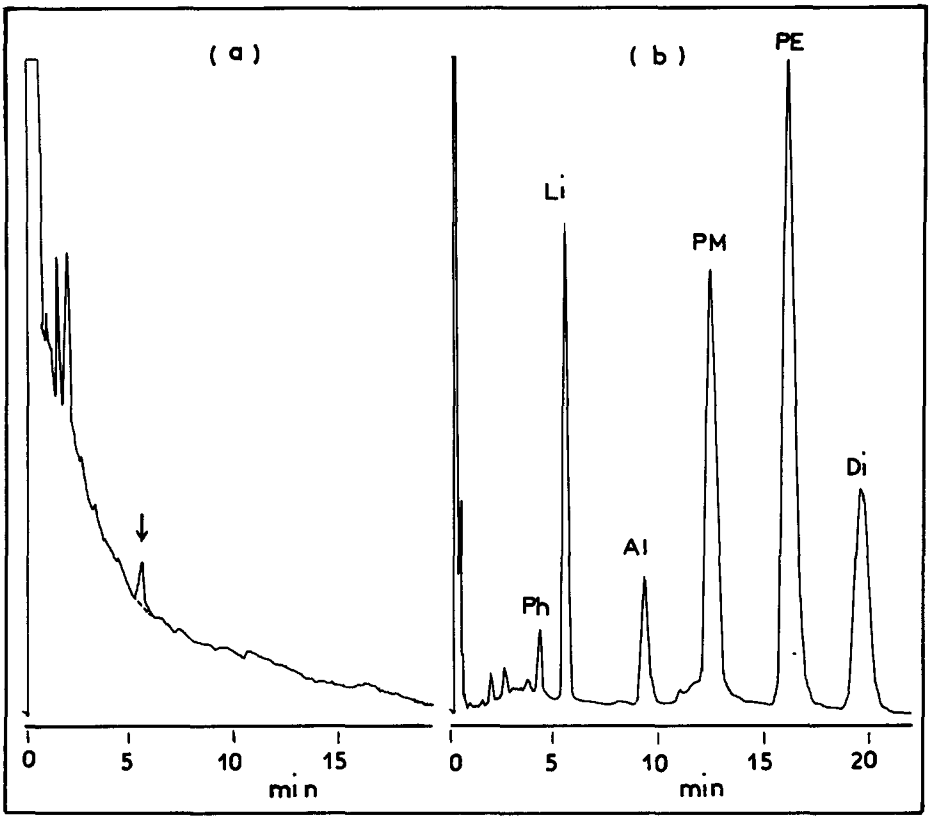

Figure 1. ECD gas chromatograms of a) Iliki lake water. The dotted line is the method blank and lindane is indicated with the arrow b) Fortified high-purity water with 0.1 µg/L lindane (Li) and Aldrin (Al), 0.2 µg/L dieldrin (Di), 1 µg/L phorate (Ph), 2 µg/L parathion methyl (PM) and parathion ethyl (PE).

Acknowledgments. The author thanks the Ministry of Environment, Department of Waters, for supporting the project and Katherine Bourou for her assistance in processing samples.

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Received February 23, 1993; accepted June 30, 1993.