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Determination of Pentachlorophenol in Environmental Samples of the S. Euboic Gulf, Greece

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Pentachlorophenol (PCP) has been widely used as a general biocide and most commonly used as wood preservative. Presumably PCP is one of the most persistent and widespread environmental pollutants today. Marine environment is very often polluted with PCP either through direct discharge of PCP contaminated effluents or indirectly through other anthropogenic activities, like storage of PCP treated timbers in coastal areas. The toxicological mode of PCP action involves the uncoupling of mitochondrial oxidative phosphorylation and thus discharging the chemiosmotic gradient formed by electron transport to drive the phosphorylation of ADP (Ahlborg *et al.* 1974, Mitchell 1961). Since PCP is an oxidative phosphorylation uncoupler, it is toxic to a variety of aerobic organisms.

Numerous articles have been published about the accidental release of PCP to the aquatic environment and massive fish kills. Furthermore, sublethal water concentrations of PCP are expected to have adverse effects on critical biological functions of water species. In mammalian species, PCP because of its extensively described intrinsic properties, can be metabolised to tetrahydroquinone (TCH). This metabolite has also been detected after the metabolism of PCP by human microsomal enzymes (Juhl *et al* 1985). TCH in contrast to PCP exhibits genotoxic properties; it binds to DNA, causes strand breaks to DNA and is more toxic to human fibroblasts than PCP (Witte *et al* 1985).

The aim of this project was to study a coastal area located in the South Euboic gulf, in central Greece, in respect to PCP pollution. PCP pollution of the area was suspected due to storage of treated timbers, imported from African countries.

MATERIALS AND METHODS

Bivalve, sediment and water samples were taken from the studied area where a timber elaboration plant is located and analysed for PCP

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contamination. The samples were collected during the time period starting from December of 1995 to March of 1997 and analysed following the developed analytical procedure. Mussel samples consisting of wild populations found in the studied area, both from the site of heavy timber accumulation (sampling site 1) and from the mouth of the gulf (sampling site 2) approximately 300m from the timber storage area, and were taken for the determination of PCP contamination. Samples of water and sediment were also taken from the same sites. They were transferred directly to our laboratories and kept properly until analysis.

The initial extraction of PCP was based on the change of PCP partitioning between the aqueous and the non polar organic phase according to the pH changes. Mussel tissues were separated from the shells before thawing, chopped into pieces with scissors and homogenised with an ultra turrax homogeniser at medium load for 2 minutes. 3 g of the homogenate were weighed directly into an Erlenmeyer flask with Teflon lined screw cap and 30 ml of concentrated HCl were added. The homogenate was digested overnight under simultaneous hydrolysis of PCP conjugates and neutralisation of phenolate anion that might be present (Stark 1969). Afterwards the specimen was transferred into a 100 ml separatory funnel and extracted twice with 30 ml of hexane. The organic phase was filtered through 3 g of anhydrous sodium sulphate pre-washed with hexane and placed in a folded Whatman filter paper. The filtrate was collected into a 100 ml round bottom flask and concentrated to approximately 5 ml by using a rotary evaporator. PCP in the hexane concentrate was extracted with 5 ml solution of 1 M KOH. The aqueous phase was collected and the pH was adjusted from alkaline to approximately pH 2 with 6-8 drops of concentrated sulphuric acid. Further extraction and clean up was achieved using the solid phase extraction (SPE) technique on the non polar material C_{18}. The sample was passed through a preconditioned C_{18} cartridge, suitable for the clean up of specimens of pH 2. The cartridge was air dried with an air pump for at least 3 hours and the PCP was eluted with 1 ml of ethyl acetate, final volume. PCP concentration was expressed as µg PCP per kg of mussel wet weight. For the fortification experiments the PCP standard solution was added prior to addition of the acid.

3 g of sediment sample were weighed after removing the small stones and 30 ml of HCI were added and left overnight. The sample was then extracted twice with 30 ml of hexane and the organic phase was separated through centrifugation and processed for the determination of PCP as described in the case of mussel samples. PCP concentration was expressed as µg of PCP per kg of sediment or as µg of PCP per kg dry weight, as determined for each sample. The water samples were analysed following the SPE technique also and under the same chromatographic conditions (Miliadis and Machera 1996).

The reagents used were of pesticide residue grade purity. The PCP analytical grade standard was purchased from the Institute of Organic Industrial Chemistry, Poland. Concentrated stock solutions of PCP were prepared in ethyl acetate; dilutions for GLC analysis and for the recovery experiments were also prepared in ethyl acetate.

For the chromatographic analysis the EPA method 8040 was followed. GLC was carried out on a Varian 3700 gas chromatograph equipped with an electron capture detector. Column temperature was set at 175°C, injector at 220°C and detector at 250°C. The Nitrogen carrier gas flow rate was 35 ml/min. A glass column, 2 m long and 2 mm internal diameter, packed with the specific for PCP material 1% SP 1240 DA on 100-120 mesh Supelcoport, was used.

For the water samples found contaminated with PCP, GLC analysis was also performed on a Fisons HRGC, Mega 2 series, equipped with an electron capture detector and a DB-5 column, 15 m long, 0.25 mm internal diameter and film thickness of 0.25 µm. The injector was in the splitless mode with the split closed for 50 sec at 210°C. The temperature program used was 50 to 230°C (5 min) with a rate 20°C/min. The carrier gas was He and the injection volume was 1 µl.

In order to confirm and quantify PCP in a given sample, the following criteria had to be fulfilled: a) A peak should appear at the same retention time as the PCP standard. In addition, the identity of the peak was confirmed through addition of PCP standard to a portion of the sample extract. The shape of the peak should not be disrupted and remain the same. b) The peaks should have a signal - to - noise ratio of greater than 5 to 1. c) The relative factors, evaluated as the ratios of the peak height of PCP in the sample extract and the peak height of the PCP standard solution, analysed under identical conditions, should agree within ± 15%. d) Reagent blanks processed with the samples should be free of PCP contamination and e) Control samples used for the fortification experiments should not have a quantifiable concentration of PCP, i.e. any coeluting peak should be of height significantly lower to that corresponding to PCP limit of detection.

RESULTS AND DISCUSSION

The response of the detector was found linear for PCP in the range of 0.01 - 1.0 ng (correlation coefficient: 0.999, intercept: -0.23, slope: 114.8). The recovery of PCP in the cases of mussel and sediment fortification, that are shown in Tables 1 and 2, varied from 100-112% for mussels and 88-93% for sediment. The statistically determined detection limit for PCP was found equal to 1.39 µg/kg in mussels and 2.3 µg/kg in sediment. The recovery experiments were performed by fortifying control mussels.

Table 1. Recovery of PCP from fortified mussels.

Fortification levels (μg/kg)	Concentration detected (μg/kg)					Mean ± SD	Recovery (%)	RSD (%)
0	1.7	0.5	1.2	0.9	0.6	0.98±0.49	-	-
5	5.1	5.4	6.0	5.9	5.5	5.58±0.37	111.6	6.6
10	11.6	10.3	10.0	10.7	11.2	10.8 ±0.65	108.0	6.0
20	18.3	18.3	23.3	20.8	21.3	20.4 ±2.13	102.0	10.4
50	43.3	53.3	48.3	51.9	52.9	49.9 ±4.20	99.8	8.4

As such, mussels purchased from the market were used, in which PCP concentration detected was found lower than the statistically determined detection limit. These very low concentrations of the control mussels are however reported in Table 1 as zero fortification level. The respective control sediment samples, on which fortification was performed, however contained no PCP detectable residues, as shown in Table 2.

Table 2. Recovery of PCP from fortified sediment.

Fortification levels (μg/kg)	Concentration detected (μg/kg)					Mean ± SD	Recovery (%)	RSD (%)
0	-	-	-	-	-	-	-	-
10	8.0	9.3	9.3	8.3	9.2	8.8±0.62	88.0	7.0
20	18.3	16.0	20.0	18.9	19.0	18.4 ±1.49	92.0	8.1
50	46.6	46.6	43.2	47.8	48.6	46.6±2.06	93.2	4.4

The analytical procedure followed in the present work, especially for the extraction step, is based on the principles of the method applied for other matrices (Borsetti 1980, Changes in methods AOAC 1985). For the clean up of the PCP the principle of the method as described by Baker (Baker EN - 502) was followed. The followed procedure has similar sensitivity to other methods applied on different matrices, as for example in the case of urine samples where the validated limit of detection was 5 ppb (Edgerton and Moseman 1979). In the case of milk and blood samples, the lowest detected concentration was equal to 0.01 and 0.05 ppm, respectively (Borsetti 1980). Finally the lowest detectable concentration for gelatine was 10 ppb (Changes in methods AOAC 1985), for sandy soil 1.65 ppb and for fish 9.8 ppb (Stark 1969).

The detected concentrations of PCP in samples of wild mussels, sediment and water from the studied area are shown in the Table 3. The determined PCP concentrations in the environmental samples i.e. mussels, sediment and sea water during the 15 months of the sampling period indicate a direct correlation of the PCP pollution levels and the timber incidence in the studied area.

Table 3. Pentachlorophenol concentration in environmental samples of the studied area.

Month of sampling	Days after the latest timber entrance	PCP concentration					
		in mussels (μg/kg)		in sediment (μg/kg)		in water (μg/kg)	
		site 1	site 2	site 1	site 2	site 1	site 2
12/95	5	-	38.3	91(196)[1]	53(103)	6.0	0.1
02/96	3	-	75.5	77(106)	-	2.5	0.6
04/96	continous entrance for 20 days	745	33	101(125)	-	8.8	-
05/96	42	-	6	-	-	0.1	0.1
05/96	continous entrance for 10 days	301	-	371(909)	-	4.0	0.2
06/96	10	16	-	74(155)	-	3.2	0.2
07/96	10	22	9	99(205)	-	0.2	N.D.
11/96	20	-	9.7	55(101)	-	1	0.1
12/96	3	409	41.5	50(160)	-	8	0.3
2/97	3	-	40.0	170(420)	-	1	0.3
3/97	2	-	-	67(174)	-	18	-

1: values in parentheses corresponds to μg PCP/kg dry weight
N.D.: Not detected

As is shown in Table 3, in the samples collected 42 days after the termination of timber entrance, the PCP levels were under or very close to the detection limit for water and mussels, which is in agreement with the reported degradation of PCP in sea water and sediment. In sea water 75% of the initial PCP concentration has been reported to degrade in 1080 hrs, whereas the proposed half-life in sediment has been reported to be 5500 hrs (Shiu *et al* 1994). In addition to the relatively high decomposition rate of PCP in sea water the local phenomenon of high and low tides, that is observed in the studied area, must be taken into consideration for the estimation of the expected time for water clearance.

The detoxification of the mussels is also seen to be nearly complete 42 days after the entrance of the treated timbers. This finding is in agreement with the results observed during a depuration experiment performed in our laboratory, where the determined PCP levels in the contaminated mussels were back to the control levels 24 hrs after exposure to clean synthetic sea water. PCP detoxification in bivalves is reported as a quite fast process and 95.2% was depurated by oysters 13 hrs after exposure to clean sea water. In general, PCP is both absorbed and eliminated rapidly by many aquatic organisms. In the case of Pacific oysters PCP has been found to be slowly absorbed but rapidly depurated (Shofer and Tjeerdema 1993) which is in agreement with our results. PCP and other phenolics are often detoxified in fresh water fish by sulphate and/or glycoside conjugation (including both glucoronidation and glucosidation) (Gates and

Tjeerdema 1993). Since the ability of marine molluscs to biotransform PCP appears to be limited the principal route of detoxification may be through rapid depuration (Shofer and Tjeerdema 1993). Thus very low concentrations of PCP metabolites are expected to be present in mussels. However the possible conjugates of PCP which are also expected to be of low extent in bivalves are detected as free PCP after the hydrolysis of the samples with HCl and transformation of the PCP conjugates to the non polar PCP form.

In the case of sediment, PCP contamination found in our study seems to be a more stable condition. The relatively high concentrations temporarily observed were directly correlated to the continous entrance of PCP treated timbers to the coastal area, followed by direct deposition and decomposition of bark and other wood pieces.

The estimated environmental fate of phenols, and in particular of PCP, has extensively been described in the literature, based mainly on the physical-chemical properties of the compounds (Shiu *et al* 1994). The phenols may exist in dissociated and non dissociated form in aqueous solution, the proportion being determined by pH and ionic strength of other ionic species. As pH increases, the tendency of dissociation increases especially for phenols of relatively low pKa such as PCP (pKa = 4.75). The net effect is not to enhance transport from air to soil and water, to reduce partitioning from water to sediment and to cause a slight overall reduction in persistence. The bioconcentration factor of PCP in activated sludge has been found to be 3.04 and in fresh water fish species from 0.09 to 3.2 (Shiu *et al* 1994).

At low pH the dissociation is slight and PCP behaves similarly to hydrophobic chemicals. At pH 5.1 the capacity of water for PCP increases, thus the water compartment becomes more important. If discharged to water, there is less water to sediment transfer because of the reduced apparent hydrophobicity and the residence time decreases. Further increases in pH to 6 and 7 causes these effects to be more pronounced. If discharged to water, there is reduced persistence because of reduced transport to sediments. According to the formula $I=10(^{pH-pKa})$ giving the ratio I of ionic to non-ionic fraction of PCP the I value for the sea water of pH=7 is equal to 178. The respective fraction of the ionic form X_i according to the formula $I/(I+1)$ is equal to 0.994 and the fraction of the non-ionic form X_n according to the formula $1/(1+I)$ is equal to 0.006. Thus it is obvious that the ionic form of PCP is dominating in the sea water, which modifies drastically the expected bioconcentration factor as it can be estimated from the respective log Kow=5.18 value (Sangster 1993).

In conclusion, the developed analytical procedure presents satisfactory sensitivity, recovery and repeatability. In addition the “uncomfortable” steps of boiling the samples in concentrated acid or the use of highly hazardous chemicals like diazomethane, were avoided without any negative effects on the quality of the method. The use of the SPE allowed us to avoid the liquid - liquid extraction for the clean up, which makes the whole procedure more friendly to the user and to the environment and more time saving. According to the analytical results obtained from the samples of the studied area it can be concluded that the PCP concentrations detected in mussels, sediment and water are directly correlated to the incidence of timber entrance to the coastal area. The detected PCP pollution is locally restricted and of limited persistence under the specific conditions of the area. However the correlation of the PCP levels to seasonal alternations and the testing on bioindicator organisms will present more reliable information about the biological condition of the ecosystem.

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