Pak. J. Sci. Ind. Res. 2007 **50**(5) 321-326

Level of Organochlorine Pesticides and Polychlorinated Biphenyls in Shellfisheries and Flounder Eggs at Virginia Beach Using Matrix Solid Phase Dispersion

Alia B. Munshi[a], Gregory D. Boardman[b], George J. Filck[c] and Hina A.Siddiqi[a]*

[a]Centre of Environmental Studies, PCSIR Laboratories Complex, Karachi -75280, Pakistan
[b]Department of Civil and Environmental Engineering, 417 Durham Hall (0246),
Virginia Tech, Blacksburg, VA 24061, USA
[c]Department of Food Science Technology, Virginia Tech, Blacksburg, VA 24061, USA

(received October 7, 2006; revised September 1, 2007; accepted September 8, 2007)

Abstract. Concentrations of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) including ΣDDTs, Σchlordanes, ΣBHCs, dieldrin, heptachlor epoxide etc were measured in the tissues of different shell fishes and flounder eggs of River James at Virginia Coast, USA. PCBs were the most predominant contaminants, followed by Σchlordanes, ΣBHCs, ΣDDTs, and other OCPs. Concentration of OCPs decreased by an order of magnitude during the last decades in this region; nevertheless, the concentration of PCBs and OCPs in shell fishes are still elevated. Concentrations of organochlorines were highly correlated with one another, and were in the range of a few to several ng/g on a wet weight basis. In the tissue of shell fishes, the sum of ΣOCPs ranged from 193.5-665.53 ng/g, predominated by Σchlordanes. ΣPCB had an overall range of 287.7-28207.9 ng/g and were predominated by ΣAroclor 1248.

Keywords: endocrine disrupting chemicals; organochlorine pesticides; polychlorinated biphenyls; shellfishes; flounder eggs

Introduction

Organochlorine pesticides are generally, highly toxic to aquatic organisms. The legislation provides that their concentration in sediment/shellfish/fish must not increase significantly with time.

Organochlorine pesticides tend to be highly bioaccumulated by aquatic organisms; their high concentrations or of their residues in marine mammals have been suggested as the cause of pathological changes and reproductive failures in Baltic sea lions, seals and Beluga whales (Zakharov and Yabloko, 1990).

Organochlorine compounds (pesticides and PCBs) are also known as endocrine disrupting chemicals (EDCs) (Portelli *et al.*, 1999) having ability to disturb the normal hormonal systems of animal species through mimicking or blocking natural hormones or by interfering with their production and metabolism (De Jager and Andrews, 2000).

All of the EDCs tested so far are toxic to marine animals at levels far below the recommended application rates. Most pesticides, particularly the chlorinated hydrocarbons, have a toxic effect on marine shellfish (Munshi *et al.*, 2004). Oysters exposed to minute concentrations of agricultural chemicals show abnormal pumping activity, decreased shell growth and significant mortality during summer. The affected animals when returned to clean water, soon recovered from all visible signs of damage. Oysters exposed to DDT at levels of 1 to 1,000 ppb (μg/liter) show a progressive decrease in shell deposition as compared with controls (Fisk *et al.*, 1998) Environmental pollution by DDT at levels as low as 0.001 ppm causes marked reduction in oyster growth. Molluscs and fish concentrate and store organochlorine pesticides at levels many thousand times greater than that present in their environment. Some pesticides caused damage at the lowest levels tested when the exposure was sufficiently long (Falandysz *et al.*, 2001).

Applications of pesticides inevitably lead to residues in soils which may evaporate to the air or be washed into watercourses, causing contamination of marine environment. In the early 1990s, the World Health Organization estimated that 3 million people a year suffered from acute pesticide poisoning with as many as 200 000 of them dying, most of them being in the developing world, where village conditions virtually prohibit safe use of the dangerous pesticides.

Protection and preservation of marine environment from possible adverse effects of agricultural chemicals is as important as the search for safe pesticides for improving the quality and

*Author for correspondence; E-mail: kmystry@hotmail.com

quantity of agricultural production. It does not imply that the widespread use of pesticide formulations automatically constitutes a serious threat to marine life.

The objective of the present study was to maintain at its optimum level the production of wholesome and economically valuable marine plants and animal products.

Materials and Methods

Sample collection. Shell fishes were procured along the Virginia Beach, USA (Fig. 1), and their biometric data were recorded. Tissue samples from different organs were dissected, wrapped in aluminum foil and stored in a deep freezer (-20 °C) until analysis.

Sample analyses. Samples were thawed (30 specimen of each shellfish), cut into small pieces and chopped to make a fine minced sample. Finely chopped frozen tissue was homogenized in glass mortar and pestle and weighed in a small weighing boat 0.5 g of tissue was spiked with surrogate standard and homogenized with surface modified bonded silica sorbent (C_{18}) using a mortar and pestle. The sample/carridge C_{18} and surrogate mixture were transferred to a syringe barrel column and extracted with methylene chloride through Bond Elut SPE Florisil-Cartridges using SPE vacuum at pressure 2.5 mm of Hg.

Prior to injecting the internal standard, an evaporator with the volume of 1ml/m was added. Surrogate standard was spiked initially in one of each five samples and its recovery

Fig. 1. Map of Virginia Coast along with James River and Chesapeake Bay.

was calculated for a mixture of pesticides for the preinstrumental analysis. At the end, before running autosampler internal standard was added for quality assurance of GC. At a time 12 columns were eluted with 25 ml methylene chloride; eluant was evaporated and transferred to approx. 2 ml hexane. Finally all extracts were concentrated under a gentle stream of nitrogen.

Instrumentation. Concentration level of PCB and OC Pesticides was determined, using Agilent 6890 Gas chromatograph (GC) equipped with micro-electron capture detector (μECD); temp. 350 °C; mode: constant column + makeup flow; combined flow: 60ml/min; make up gas type: nitrogen; Inlet: operated in split less mode; initial temp: 200 °C; pressure:17.38 psi; purge flow:15ml/min; total flow: 19.2 ml/min; Oven: initial temp: 100 °C; hold time: 5 min; ramp at 4.0 °C/min to 220 °C; two capillary columns were used for initial injections (calculations) and for confirmation injections (validate initial injection of pesticides).

a) Initial injections: RTX5 w/Integra Guard, 30 m x 0.25 mm x 250 μm ID x 0.25 μm film thickness; constant pressure: 17.38 psi; nominal initial flow: 33 cm/s.

b) Confirmation injection: RTX35 w/Integra Guard, 30 m x 0.25 mm x 250 μm ID x 0.25 μm film thickness; constant pressure: 17.38 psi; nominal initial flow: 33 cm/s.

Different chromatographic columns were used for quality assurance. The identification was carried out by matching the retention time (both absolute and relative to the internal standard) obtained from the sample and the working standard solutions injected in the same sequence of analysis and over the runs with the two GC columns.

The quantification of each compound was carried out by comparison of its peak area ratio to the internal standard in the sample run with that obtained by injecting a working standard solution at concentration comparable to that of sample.

Performance and quality control. All the glasswares were washed. No rubber or plastic items other than PTFE were used. Method "blanks" were periodically run to ascertain absence of contamination.

Internal spiking and reagent blank determined the recovery values which ranged 60-149% and 92-157% for OCPs and PCBs, respectively. Generally, precision for pesticide residual analysis varies up to 10; therefore, the recovery of any metabolite is expected to be up to 200 which is also acceptable in such specific analysis. Precision was assured through multiple analyses of spiked samples (every set of 5 samples has one spiked sample with 5 multiple analysis) and ranged 1.1-11.9% for different compounds. The limit of detection

(LOD) was calculated on the basis of RSD percentage which was 0.001-0.01μg/g or 0.1- 1ng/g; limit of reportable amount was obtained by multiplying the value with 3, which was 0.003 μg/g or 0.3ng/g for PCB congeners and for OCPs.

Results and Discussion

Chlorinated pesticide-spiked fish tissue sample was repeatedly analyzed to determine the reproducibility, detection limits, RSD and recovery. (Table 1). Chlorinated pesticides (20) and congeners (7) of PCBs were extracted through Matrix Solid-Phase Dispersion (MSPD) and quantified using gas chromatograph. Spiking experiments were carried out to determine the recovery, precision, and limit of detection (LODs) of the method. The overall recovery was above 90-260% in the spiked tissue sample at 4.00 ng/g level. The detection limits for chlorinated pesticides ranged from 0.1 to 1 ng/g,. In view of their recovery and removal of interference, MSPD is a reliable method for the confirmation and quantization analysis of chlorinated pesticides (Barker and Hung, 1999). It has good resolution along with best recovery of each metabolite of the pesticides and was applied in the present study. MSPD is the same Solid Phase Extraction (SPE). Σchlordane and ΣBHC were the most dominant organochlorine group in the present study. Other chlorinated pesticides, such as DDT, dieldrin, endrin and chlordane, were all present in very low concentrations in all samples of shellfish.

Table 1. List of 20 organochlorine pesticides and 7 polychlorinated biphenyls with their average recovery (ng/g)

Pesticides	Recoveries %	RSD	LOD	LOR
Organochlorine				
Aldrin	90	2.87	2.521	7.563
Alpha BHC	115	4.51	2.149	6.420
Alpha chlordane	119	6.59	1.802	5.4o6
Beta BHC	115	2.45	1.938	5.784
Delta BHC	99	7.13	2.364	7.092
Dieldrin	145	7.66	2.098	6.974
Endosulphan I	100	10.41	1.885	5.655
Endosulphan II	140	6.55	8.566	25.698
Endosulphan sulphate	60	1.50	3.697	13.482
Endrin	113	4.63	2.455	7.365
Endrin aldehyde	101	7.00	4.114	12.342
Endrin ketone	74	6.00	1.268	3.804
Gamma BHC	149	4.60	1.204	3.612
Gamma-chlordane	117	5.91	2.976	8.928
Heptachlor	118	5.59	9.060	27.180
Heptachlor epoxide	114	7.20	10.321	30.953
Methoxychlor	NR	0.43	1.516	4.548
p,p DDD	149	7.08	4.637	13.911
p,p DDE	121	14.77	5.792	17.376
p,p DDT	NR	NR	ND	ND
Polychlorobiphenyl				
Aroclor1016	122	13.92	4.641	13.92
Aroclor1221	145	20.23	6.742	20.23
Aroclor1232	92	19.72	6.574	19.72
Aroclor1242	152	21.07	7.025	21.07
Aroclor1248	108	21.62	7.206	21.62
Aroclor1254	134	22.36	7.454	22.36
Aroclor1260	157	0.00	0.000	0.00

LOD = limit of detection; RSD = relative standard deviation; LOR = limit of report; NR = no response; ND = not determined

As in the case of the OCPs, the highest concentration of ΣBHCs was present in oyster with mean concentration of 66.41 ng/g wet weight, and the lowest concentration in the crab (33.34 ng/g) (Table 2). Heptachlor was found in all shell fish with mean value of 19.79 ng/g, and maximum concentration (42.08 ng/g wet weight) in Flounder eggs with 5.82% of ΣOCPs in all shell fishes. In the analyzed samples, pattern of DDT group is quite similar, highest concentration (49.6 ng/g) present in Flounder eggs and minimum (43.42 ng/g) in white shrimp. Thus, it is apparent that River James is not receiving significant new inputs of DDT. The sum of chlordanes group (Σchlordanes) is also the most prevalent group of contaminant. The highest concentrations was found in Flounder eggs (349.59 ng/g wet weight) followed by lowest value (44.186 ng/g wet weight) in oyster.

Results of the present study show that OCPs are still among the most prevalent environmental pollutants and can be found in various environmental compartments at any time (Fig. 2). Their widespread presence is due to their extremely persistent and lipophilic nature enabling them to bioaccumulate in the adipose tissues of fishes resulting in the enrichment throughout the food chain (De Voogt *et al.*, 1990); prolonged exposure to these pollutants can interfere with normal physiology and biochemistry (Picard *et al.*, 2003). The

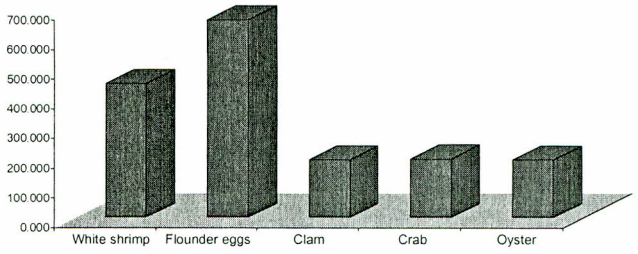

Fig. 2. Total OCP concentration in different shell fishes.

Table 2. Concentration of organochlorine pesticides (ΣBHCs) in shell fisheries and flounder eggs (ng/g)

Compound	White shrimp	Flounder eggs	Clam	Crab	Oyster
alpha-BHC	14.671	14.372	8.895	8.791	8.521
beta-BHC	2.699	2.644	6.928	7.067	7.067
delta-BHC	16.196	18.200	5.148	5.251	5.251
gamma-BHC	19.089	18.699	12.267	12.230	12.230
heptachlor	25.730	42.082	10.393	10.379	10.379
aldrin	47.755	27.054	10.466	10.898	10.676
heptachlor epoxide	81.420	98.410	10.026	11.309	9.899
alpha-chlordane	35.353	31.959	10.087	10.724	10.067
gamma-chlordane	6.961	6.819	8.895	9.379	9.073
endosulphan I	22.413	26.086	10.878	10.748	10.748
dieldrin	24.365	40.392	10.485	10.373	9.950
p,p-DDE	25.223	31.761	10.269	9.776	9.776
endrin	11.880	21.065	8.481	8.650	8.650
endosulphan II	20.183	28.794	8.707	9.568	8.881
p,p-DDD	6.786	6.648	24.078	24.559	24.559
endrin aldehyde	9.249	32.110	9.765	9.520	9.520
p,p DDT	11.417	11.184	12.033	12.273	12.273
endrin ketone	11.061	36.953	11.016	11.236	11.236
methoxychlor	59.001	170.321	4.674	4.768	4.768
endosulphan sulphate	ND	ND	ND	ND	ND

ND = not determined

composition of lipids can also influence the bioaccumulation of organochlorine compounds (Kawai *et al.*, 1988).

PCBs congeners were determined in shell fish tissue from Virginia Coast (Table 3). Summed concentrations of 7 chlorobiphenyl congeners (ΣPCBs) ranged from 287.7 ng/g to 29207.9 ng/g wet weight with the lowest concentrations in the crab and highest concentration in white shrimp. The levels of PCB congeners 1221, 1232, 1248 and 1254 in the white shrimp were markedly higher than those found in other shell fishes due to different points of collection and significantly higher lipid content. Concentration values of the PCB congeners reveal that Aroclor 1248, Aroclor 1245 and Aroclor 1232 predominate, up to 31.65%, 23.03% and 16.56% of ΣPCBs, respectively, followed by Aroclor 1221 and Aroclor 1016 (15.33% and 0.47%, respectively, being the lowest ratio of ΣPCB). Concentration of PCBs was in the same range in crab and clan. (Fig. 3). It was minimum in crab and maximum in white shrimp. The lowest amount of Aroclor 1016 (63 ng/g) was in clam and 83.9 in oyster but was not found in white shrimp, flounder egg and crab. In all cases, the minimum concentration of ΣPCB was found in crab (287.7 ng/g) and maximum in white shrimp (29207.9 ng/g). The organochlorine contents reported in this study fell in the lower range of those reported as global comparators and probably reflect the chronic contamination of the area

Table 3. Concentration of PCBs in shell fisheries and flounder eggs (ng/g) from Virginia Beach, USA

Compound	White shrimp	Flounder eggs	Clam	Crab	Oyster
PCB-1016	<LOR	<LOR	63.0	<LOR	83.9
PCB-1221	4339.5	101.5	116.1	78.8	62.8
PCB-1232	4592.1	271.3	45.4	74.0	92.1
PCB-1242	3780.0	<LOR	42.4	43.6	97.1
PCB-1248	9439.4	69.8	38.9	91.3	57.8
PCB-1254	7056.9	<LOR	<LOR	<LOR	<LOR
PCB-1260	<LOR	<LOR	<LOR	ND	<LOR
Total PCBs	23114.9	<LOR	<LOR	<LOR	<LOR

LOR = limit of report

rather than the influence of coastal discharges whereas, the pattern of PCB congener was the same in clam, crab and oyster.

Indeed, these congeners are present in higher proportions in industrial PCB formulations (Schulz *et al.*, 1989) and seem to be responsible for their persistence and bioaccumulative properties. Aroclor 1221,1232,1248 and 1254 represent the predominant congeners in white shrimp and flounder eggs; other congeners were not present at more than 10-12% of ΣPCBs. PCBs with higher chlorine content are usually

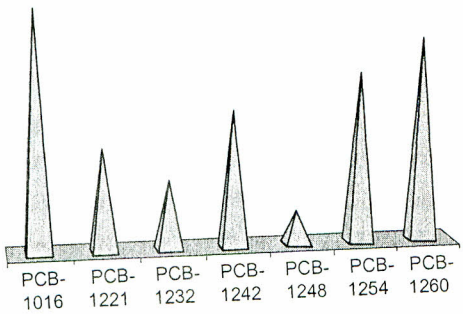

Fig. 3. Concentration of PCBs in different shell fishes.

more difficult to metabolize; hence, their persistence or bioaccumulation is greater (Safe, 1994). It is unclear why there is such a marked difference in pollutant concentrations in different fishes having almost similar ecological characteristics. This variability can be the result of different feeding habits and/or dissimilar ability of different species to accumulate pollutants.

In fact, for ΣPCBs and ΣOCPs the mean level on wet weight basis of contamination in the examined tissues always have a linear relationship with their mean lipid content (Aguilar *et al.*, 1999). ΣPCBs and ΣOCPs varied in male and female fishes (Fig. 2 & 3).

Conclusion

The levels of ΣPCBs and ΣOCPs in tissues of different shell fishes (a top marine predator) from River James (Virginia Coast) were found to be lower than those found in other parts of the world as well as in comparison to other marine organisms at the lower stages of the food chain, the levels of persistent, lipophilic organochlorine contaminants did not increase in this part of Virginia Coast.

It was found that the concentration of organochlorine contaminants in shellfish decreases in the order flounder eggs > white shrimp > crabs > oyster > clam > and with respect to PCBs, the profile was white shrimp > eggs of flounder > clam > oyster > crabs. Factors affecting the level of contamination in shell fishes included the fat content and the metabolic characteristics of each species. Furthermore, the higher concentration of organic contaminants measured in these samples indicate that anthropogenic activities, such as industrialization and agricultural practices, have not much affected the water quality of this area. Their relatively smaller concentration reflect the distance from anthropogenic source of contamination, atmospheric sources of pollution and large dilution factor.

Acknowledgement

This study was conducted at the Virginia Polytechnic Institute and State University, Virginia and funded by the Council for International Exchange of Scholars (CIES) under the Fulbright Scholar Programme on behalf of the United States Department of State.

Authors would like to thank all staff members of USEF/P and CIES for providing the opportunity by awarding the Fulbright scholarship.

Thanks are due to Robert M. Lane, Jean Cobb (Pesticide Residues Lab.) for their continued help and expert opinion at every stage of this study. Dave Kunn and Amy K. Cheatham are also appreciated for their sincere efforts for this study.

References

Aguilar, A., Borrell, A., Pastor, T. 1999. Biological factors affecting variability of persistent pollutant levels in cetaceans. *J. Cetacean Res.Manag.* **1:** 83-116.

Barker, S., Hung, N.N. 1999. *Matrix Solid-Phase Dispersion (MSPD): New Methods for the Rapid Extraction and Analysis of Pesticides in Food.* Varian Sample Preparation Products (MSPD method), Harbor City, California, USA.

De Jager, L.S., Andrews, A.R.J. 2000. Development of a rapid screening technique for organochlorine pesticides using solvent microextraction (SME) and fast gas chromatography (GC). *Analyst* **125:** 1943-1948.

De Voogt, P., Wells, D.E., Reutergardh, L., Brinkman, U.A.Th. 1990. Biological activity, determination and occurrence of planar, mono- and di-ortho PCBs. *Int. J. Environ. Anal. Chem.* **40:** 1-46.

Falandysz, J., Strandberg, L., Puzyn, T., Gucia, M., Rappe, C. 2001. Chlorinated cyclodiene pesticide residues in blue mussel, crab, and fish in the Gulf of Gdansk, Baltic Sea. *Environ. Sci. Technol.* **35:** 4163-4169.

Fisk, A.T., Norstrom, R.J., Cymbalisty, C.D., Muir, D.C.G. 1998. Dietary accumulation and depuration of hydrophobic organochlorines: Bioaccumulation parameters and their relationship with the octanol/water partition coefficient. *Environ. Toxicol. Chem.* **17:** 951-961.

Hansen, P.D., Von Westernhagen, H., Rosenthal, H. 1985. Chlorinated hydrocarbons and hatching success in Baltic Herring spring spawners. *Mar. Environ. Res.* **15:** 59-76.

Kawai, M., Fukushima, M., Miyazaki, N., Tatsukawa, R. 1988. Relationship between lipid composition and organochlorine levels in the tissues of striped dolphin. *Mar. Pollut. Bull.* **19:** 129-133.

Munshi, A.B., Schulz -Bull, D., Schneider, R., Zuberi, R. 2004. Organochlorine concentrations in various fish from different locations at Karachi Coast. *Mar. Pollut. Bull.* **49:** 597-601.

Picard, A., Palavan, G., Robert, S., Pesando, D., Ciapa, B. 2003. Effects of organochlorine pesticides on maturation of starfish and mouse oocytes. *Toxicol. Sci.* **73:** 141-148.

Portelli, M.J., De Solla, S.R., Brooks, R.J., Bishop, C.A. 1999. Effect of dichlorodiphenyl trichloroethane on sex determination of the common snapping turtle (*Chelydra serpentina serpentina*). *Ecotoxicol. Environ. Saf.* **43:** 284-291.

Safe, S.H. 1994. Polychlorinated biphenyls: Environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit. Rev. Toxicol.* **24:** 87-149.

Schulz, D.E., Petrick, G., Diunker, J.C. 1989. Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography-electron capture detection. *Environ. Sci. Technol.* **23:** 852-859.

Zakharov, V.M., Yablokov, A.V. 1990. Skull asymmetry in the Baltic grey seal: effects of environmental pollution. *Ambio* **19:** 266- 269.