Bull Environ Contam Toxicol (2009) 83:880–884
DOI 10.1007/s00128-009-9833-2

Levels of Organochlorine Compounds in the Mediterranean Blue Mussel from the Adriatic Sea

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Received: 17 March 2009 / Accepted: 8 July 2009 / Published online: 22 July 2009

Abstract The distribution of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) was investigated in Mediterranean blue mussel collected at four locations in Mali Ston Bay few times a year in 2005–2007. OCPs were found in all samples and levels ranged between 0.07 and 7.58 ng g^{-1} dry wt. Levels of PCBs ranged between 0 (below detection limit) and 21.55 ng g^{-1} dry wt. For most analyzed compounds there were no significant level changes between the 3 years. Exceptions are decreased levels of β-HCH, DDD, and PCB-138 and increased levels of γ-HCH and DDT in 2007. However, mussels from this area are applicable for human diet.

Keywords PCBs · Organochlorine pesticides · Mediterranean · Marine pollution

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are chlorinated aromatic compounds of anthropogenic origin, and because of their good physicochemical properties they had been used for many years in different industries, agriculture, and public health (WHO/UNEP 1972, Voldner and Li 1995). Being highly lipophilic and persistent, they accumulate in the adipose tissue of animals and humans and pose a threat for human health. Although their use was banned or restricted in the 1970s, they still persist in all parts of the environment.

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The Mediterranean blue mussel (lat. *Mytilus galloprovincialis*) is the most widespread shellfish species in the Adriatic Sea and an important foodstuff. As filter feeder, the mussel has a great capacity of accumulating organic contaminants, and it is frequently used as a biomonitor of environmental pollution. Because of wide spatial distribution and weak mobility, pollution data can easily be compared.

In the Mali Ston Bay, this shellfish has been cultivated for several centuries. The aim of this study was to investigate variations of 17 PCB congeners and 7 OCPs in the edible tissue of mussels from the Mali Ston Bay, and to establish whether there was a health risk for people consuming shellfish from this area.

Materials and Methods

Mussels for PCB and OCP analysis were collected from natural populations at four breeding farms at the Mali Ston Bay (Fig. 1) in March, May, August, and December 2005 (at location Mali Ston, samples were not collected in March due to bad weather). In 2006 and 2007, the mussels were collected in March, May, and August. Mussels were collected from a plastic/fibreglass boat by hand. Twenty individual mussels of similar shell length were collected at each sampling site, placed in plastic bags, and transported to the laboratory. The average age of all analysed mussels was 2–2.5 year (according to personal communication with workers on shellfish farm). To remove adhering detritus, mussels were scrubbed with a brush, then washed with seawater and dissected in the laboratory (Bernhard 1976). Dissected tissues were weighed, and the shell length and weight of each specimen were measured. After dissection, composite samples of mussel tissue were freeze-dried and homogenised to prepare lyophilisate. In samples collected

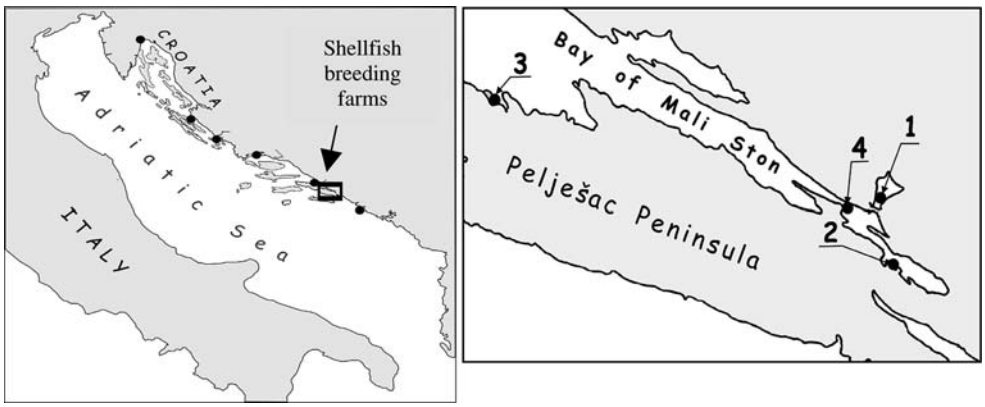

Fig. 1 Locations of the shellfish farms at the Mali Ston Bay; *1* Bistrina, *2* Mali Ston, *3* Sutvid, *4* Usko

in 2005, 2006, and 2007 water content in soft tissues varied between 85.3% and 88.1%, 85.9% and 89.8%, and 81.0% and 91.3%, respectively. The respective shell lengths varied between 6.17 and 7.30 cm, 6.27 and 7.24 cm, and between 5.65 and 8.22 cm. The respective weights of dissected tissues varied between 3.32 and 6.05 g, 3.11 and 4.80 g, and between 2.51 and 7.50 g.

Seventeen PCB congeners were analysed: PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180, PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, PCB-170, PCB-189, PCB-60, and PCB-74 (numbered according to IUPAC) and the following OCPs: hexachlorobenzene (HCB), α-HCH, β-HCH, γ-HCH (α-, β-, γ-hexachlorocyclohexanes), 1,1-dichloro-2,2-di(4-chlorophenyl) ethylene (DDE), 1,1-dichloro-2,2-di(4-chlorophenyl)ethane (DDD), and 1,1,1-trichloro-2,2-di(4-chlorophenyl) ethane (DDT).

About 1 g of lyophilisate was mixed with 2 g of sodium sulphate in a glass mortar. For extraction, 10 mL of *n*-hexane was added in the mixture and mixed until homogeneous. This procedure was repeated two more times with the same volume of hexane. The mixture was passed through filter paper (Whatman No. 1) to a preweighted test tube. The extract was evaporated to lipid residues under a gentle stream of nitrogen. Lipid residues were weighed. Lipids were dissolved in 5 mL of *n*-hexane and cleaned up with 4 mL of 96% sulphuric acid. The clean-up was repeated two more times. The solvent was evaporated to residues under a gentle stream of nitrogen. Before gas chromatography the residues were dissolved in 1.0 mL of *n*-hexane.

For high resolution gas chromatography with electron capture detector(s) (HRGC/ECD) we used two "ATI UNICAM" 610 SERIES chromatographs with ^{63}Ni detectors. The first chromatograph was used to analyse compounds simultaneously on two capillary columns ("Supelco", Bellefonte, USA): (1) 60 m × 0.25 mm, SPB-5 film thickness 0.25 μm, temperature programme 100°C, then 4°C min^{-1} to 240°C, 50 min isothermally; and (2) 30 m × 0.25 mm, SPB-1701 film thickness 0.25 μm, temperature programme 110°C, then 4°C min^{-1} to 240°C, 50 min isothermally. On the second, the compounds were separated on the capillary column ("Supelco", Bellefonte, USA) 60 m × 0.25 mm, SPB-5 film thickness 0.25 μm, temperature programme 100°C, then 10°C min^{-1} to 240°C, 80 min isothermally. The carrier gas was nitrogen. The injector and detector temperature were 250 and 270°C, respectively, and the volume of injected sample was 5 μL. Only compounds identified on all columns were evaluated. Qualitative and quantitative analyses were done by comparison with external standard.

Method recovery and reproducibility were determined with two series of samples. In each series, there were seven aliquots of homogenised samples. To five aliquots, a known amount of analysed compounds was added before extraction. Concentrations of added compounds were in the levels similar to those expected in real samples (range 1.18–11.09 ng g^{-1} dry wt). The recoveries of PCBs and OCPs were calculated after subtracting the mean levels of two non-fortified samples from the fortified ones. The recoveries ranged from 42% to 90% with relative standard deviation from 4% to 25%, depending on the compound. The determination limits for the analysed compounds were 0.03 ng g^{-1} dry wt for PCBs and 0.07 ng g^{-1} dry wt for OCPs. Performance of the analytical procedure has been validated through internal QA/QC and interlaboratory comparison.

Results and Discussion

In 2005, 15 samples were analysed and OCPs were found in all samples. PCBs 157 and 189 were found in less then 50% of samples (in seven and one sample, respectively). PCBs 60, 123, 118, 114, 156, and 170 were present in more than 70% of samples and the rest of PCB congeners were

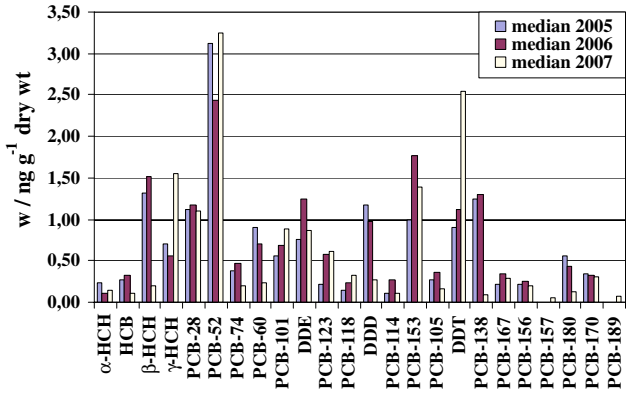

Fig. 2 Median PCB and OCP concentrations (ng g^{-1} dry wt) in Mediterranean blue mussels collected at the Mali Ston Bay in 2005, 2006 and 2007

found in all samples. The levels of PCB congeners found in all samples ranged between 0.09 and 21.55 ng g^{-1} dry wt, while of those not found in all samples ranged between 0 and 6.72 ng g^{-1} dry wt. In the same year, OCP levels ranged between 0.11 and 5.32 ng g^{-1} dry wt.

Of 12 analysed samples in 2006, PCBs 157 and 189 were found only in 5 and 4 samples, respectively. PCBs 123, 118, 156, and 170 were found in 11 samples, and all other compounds were found in all samples. OCP levels in 2006 ranged between 0.08 and 7.58 ng g^{-1} dry wt. Levels of PCBs found in all samples ranged between 0.09 and 9.45 ng g^{-1} dry wt and of those not found in all samples ranged between 0 and 4.60 ng g^{-1} dry wt.

A slightly different situation was noted in 2007, as PCB 170 was found in 11 of 12 samples, and PCB 157 in 8 samples. All other compounds were found in all samples. The levels of PCBs found in all samples ranged between 0.03 and 14.56 ng g^{-1} dry wt while of those not found in all samples between 0 and 2.23 ng g^{-1} dry wt. OCP levels in 2007 ranged between 0.07 and 6.81 ng g^{-1} dry wt.

Figure 2 shows median concentrations of compounds whose medians were above 0. In the OCP group, α-HCH and HCB had the lowest medians in all 3 years. In 2005 and 2006, the medians of β-HCH and DDT and its metabolites were the highest. In 2007, the highest medians were found for DDT, γ-HCH, and DDE.

Table 1 Concentration medians (ng g^{-1} dry wt) of organochlorine compounds determined in mussels collected at four locations of the Mali Ston Bay by sampling month

Compound	2005				2006			2007		
	March	May	August	December	March	May	August	March	May	August
α-HCH	0.23	0.36	0.23	0.21	0.11	0.29	0.09	0.13	0.21	0.13
HCB	0.23	0.43	0.34	0.30	0.28	0.51	0.35	0.10	0.14	0.10
β-HCH	1.21	1.63	1.71	0.98	0.87	1.69	1.82	0.21	0.24	0.15
γ-HCH	0.58	0.98	0.93	0.52	0.47	1.40	0.42	1.59	1.70	1.25
PCB-28	1.11	1.92	2.00	0.83	1.02	1.26	1.22	1.12	1.10	0.86
PCB-52	2.25	3.91	3.32	3.19	1.89	3.46	4.09	3.16	7.61	2.82
PCB-74	0.42	0.55	0.41	0.21	0.36	0.69	0.48	0.18	0.25	0.22
PCB-60	0.74	1.35	0.84	0.89	0.51	0.79	0.85	0.26	0.28	0.18
PCB-101	0.32	0.81	0.60	0.35	0.48	8.23	0.65	1.16	0.63	0.59
DDE	0.64	1.44	0.81	0.57	1.36	1.69	1.16	0.84	1.10	0.77
PCB-123	0.52	1.04	0.24	0.20	0.66	0.54	0.54	1.19	0.51	0.32
PCB-118	0.12	0.25	0.14	0.07	0.21	0.57	0.21	0.66	0.28	0.25
DDD	1.37	2.01	1.12	0.27	1.45	0.55	0.89	0.18	0.35	0.44
PCB-114	0.11	0.58	0.15	0.10	0.31	0.20	0.25	0.06	0.13	0.12
PCB-153	0.76	3.88	1.33	0.94	1.77	2.61	1.48	2.05	1.38	1.25
PCB-105	0.19	0.41	0.21	0.28	0.37	0.43	0.38	0.17	0.16	0.15
DDT	0.57	1.45	1.12	0.98	1.89	0.93	1.09	2.90	3.12	1.85
PCB-138	0.68	4.52	1.41	0.74	1.22	2.72	1.27	0.09	0.09	0.12
PCB-167	0.27	0.55	0.27	0.16	0.24	0.75	0.35	0.14	0.22	0.79
PCB-156	0.15	0.34	0.19	0.21	0.18	0.73	0.25	0.32	0.16	0.17
PCB-157	0.00	0.18	0.18	0.06	0.00	0.30	0.00	0.08	0.00	0.11
PCB-180	0.60	1.14	0.42	0.52	0.42	0.46	0.34	0.23	0.12	0.09
PCB-170	0.18	0.42	0.55	0.28	0.28	1.30	0.30	0.26	0.65	0.69
PCB-189	0.00	0.00	0.00	0.00	0.00	0.60	0.00	0.21	0.06	0.04

Over the 3 years, DDT showed a clear increasing trend, followed by DDE and γ-HCH, while β-HCH, DDD, and HCB decreased, and α-HCH retain similar medians.

Over the 3 years, the most abundant congeners were six indicator congeners 28, 52, 101, 138, 153, and 180, followed by PCBs 60, 74, and 170. The lowest medians were found for PCBs 157, 189, and 114.

Among all analysed compounds, a pronounced decrease in median concentrations over the 3 years was noted for β-HCH, DDD, and PCB-138, while γ-HCH and DDT showed a notable increase.

α-HCH/γ-HCH and DDE/DDT ratios are often used to see if there is a fresh input of γ-HCH or DDT in the environment. Ratios below 1 indicate fresh contamination. In 2005 and 2006, the median DDE/DDT ratio was 0.98 (range: 0.30–2.71) and 1.04 (range: 0.14–2.56), respectively. However, in 2007 this ratio decreased to 0.41 (range: 0.27–0.47).

Medians of α-HCH/γ-HCH ratios lower than 1 (0.37, 0.20, and 0.10 in 2005, 2006, and 2007, respectively) point to a fresh contamination with γ-HCH.

Table 1 shows concentration medians in mussels collected at four locations at different times of the year. Most organochlorine pesticides had the highest medians in the warmer months (May, August) and lower in the colder months (March, December). Exceptions are DDT and DDD with high medians in March 2006. PCBs showed a similar behaviour, most had the highest medians in May and/or August. Exceptions were found in 2006 for PCBs 123 and 114, and in 2007 for PCBs 101, 123, 118, 153, 156, 180, and 189, when the highest medians were in March.

Most analysed compounds show no significant changes in the levels between the 3 years. An exception are the decreased levels of β-HCH, DDD, and PCB-138 and increased levels of γ-HCH and DDT in 2007. This

Table 2 Levels of organochlorine compounds in mussels from different locations

Location	Mussel	Pollutants	Range (ng g^{-1} fat weight)	Reference
Central Adriatic Sea–Italian coast	*Mytilus galloprovincialis*	PCB-28	ND	Perugini et al. (2004)
		PCB-52	30.49–210.25	
		PCB-101	29.02–390.22	
		PCB-118	15.47–123.67	
		PCB-138	51.15–446.46	
		PCB-153	35.48–361.55	
		PCB-180	ND–36.07	
		p,p′-DDE	32.20–191.72	
		p,p′-DDD	23.72–81.35	
		p,p′-DDT	ND–32.42	

Location	Mussel	Pollutants	Range (ng g^{-1} dry weight)	Reference
Kaštela Bay–Adriatic Sea	*Mytilus galloprovincialis*	ΣPCBs (Aroclor 1254 and Aroclor 1260)	26.0–102.8	Milun et al. (2004)
		ΣDDTs	4.1–7.8	
NW Mediterranean coast	*Mytilus galloprovincialis*	PCB-52	1.10–37	Villeneuve et al. (1999)
		PCB-101	4–130	
		PCB-138	4.80–270	
		PCB-153	7.70–120	
		ΣDDTs	20–630	
		HCB	0.08–1.90	
		α-HCH	0.25–82	
		β-HCH	0.04–3.50	
		γ-HCH	0.79–3.10	

Location	Mussel	Pollutants	Range (ng g^{-1} fat weight)	Reference
Korea	*Mytilus edulis*	PCBs	17–1,000	Ramu et al. (2007)
		DDTs	21–400	
		HCB	0.31–50	
		HCHs	1.1–82	
Kentucky Lake, USA	Different mussel species	PCBs	<0.01–30	Loganathan et al. (2008)

corresponds to α-HCH/γ-HCH and DDE/DDT ratios lower than 1, which indicated a fresh input of γ-HCH and DDT in the environment.

It is important to point out that all the values obtained for mussels from the Mali Ston Bay are orders of magnitude below prescribed maximum permissible levels (MLP) for PCBs and OCPs in mussels for human use (MEHSCRC 2008; European Parliament, Council 2005). In addition, they correspond to known global pollution by persistent organic pollutants and are comparable with lower concentration ranges for the same compounds reported in literature (Table 2). Our results suggest that the levels of organochlorine compounds in mussels do not pose any threat for human health.

Acknowledgments We wish to thank Mrs Mirjana Kralj for excellent technical assistance during experimental work and Mr Dado Čakalo for language revision. This work was supported by the Ministry of Science, Education and Sports, Croatia.

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