LEVELS OF PERSISTENT ORGANOCHLORINE PESTICIDES IN EDIBLE FISH FROM THE ADRIATIC SEA

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Introduction

Organochlorine pesticides (OCP) are chlorinated organic chemicals produced and used for several decades, mainly for agriculture and public health purposes. They belong to the group of *Persistent Organic Pollutants* (POPs) which means that they have properties like lipophility, chemical stability and strong bioaccumulative nature. These properties made them a threat to animals and humans so their production and use was banned or restricted in 1970s. In spite of that, they are still found in all environmental compartments. Humans are exposed to these contaminats mainly through food, and especially marine and dairy food products.

The aim of this study was to investigate the levels of seven OCPs in edible fish species collected along the Eastern Adriatic coast (Croatia).

Materials and methods

The samples of 19 different fish species (Table 1) which belong to different families with different feeding habits (i.e. different trophic levels in marine ecosystem) were collected on 17 locations in the eastern part of the Adriatic Sea (Figure 1) in September 2007. Fish were caught by small mid-water trawl net. Sampling depth/positions were chosen according to the presence of fish echo traces identified by echo sounder. In the on-board laboratory, total length of every individual was measured and fish were dissected using stainless steel instruments on a clean glass working surface. Composite samples of muscle tissues (i.e. fillets) of each species were taken from fresh fish. Muscle samples were separated using the methods recommended by UNEP/FAO/IAEA/IOC[1], stored in aluminum foil and kept frozen at -18°C until analyses of OCPs.

Seven OCPs were analysed: 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).

Samples were analysed using a procedure reported previously[2]: extraction with *n*-hexane in a glass mortar was repeated two times. After gravimetric determination of lipid content, the extract was cleaned with sulphuric acid. The solvent was evaporated to dryness under a gentle stream of nitrogen. Before gas chromatography the residues were dissolved in 1.0 mL of *n*-hexane.

High resolution gas chromatography with electron capture detector(s) (HRGC/ECD) was done on two "ATI UNICAM" 610 SERIES chromatographs with ^{63}Ni detectors. Details about temperature programs were also reported[2]. Only compounds identified on all columns were evaluated. Qualitative and quantitative analyses were done by comparison with external standard.

The method recovery and reproducibility were determined by adding a known amount of analysed compounds to five aliquots of homogenised samples before extraction. The recoveries of OCPs were calculated after subtracting the mean levels of two non-fortified samples from the fortified ones. The recoveries ranged from 61% to 69% with standard deviation from 8% to 11%, depending on the compound. The determination limits for the analysed compounds were 0.17 ng g^{-1} fat weights for β-HCH, DDE, DDD, DDT and 0.10 ng g^{-1} fat weights for HCB, α-HCH, γ-HCH; and it was the average of all determinations based on signal to noise ratio and recovery of compounds. Performance of analytical procedure has been validated through internal QA/QC and interlaboratory comparison (participation in interlaboratory studies organized by International Atomic Energy Agency – Marine Environment

Laboratory (IAEA-MEL) – Run 435 on OCPs and PCBs). Reagent blank was used to indicate laboratory contamination and the concentration of analytes was less than determination limit.

Table 1: List of fish species and sampling locations

English name	Latin name	Family	Sampling location number
Annular seabream	*Diplodus annularis*	Sparidae	11
Blue whiting	*Micromesistius poutassou*	Gadidae	11
Bogue	*Boops boops*	Sparidae	12
Brown comber	*Serranus hepatus*	Serranidae	12
Chub mackerel	*Scomber japonicus*	Scombridae	4, 13
Comber	*Serranus cabrilla*	Serranidae	9, 15
Common stingray	*Dasyatis pastinaca*	Dasyatidae	16
European anchovy	*Engraulis encrasicolus*	Engraulidae	2, 3
European barracuda	*Sphyraena sphyraena*	Sphyraenidae	12
European hake	*Merlucius merlucius*	Gadidae	6, 12, 15
Horse mackerel	*Trachurus mediterraneus*	Carangidae	3, 7, 12
Horse mackerel	*Trachurus trachurus*	Carangidae	10
John dory	*Zeus faber*	Zeidae	6
Pandora	*Pagellus erytrinus*	Sparidae	1
Picarel	*Spicara flexuosa*	Centracanthidae	3, 15
Red gurnard	*Aspitrigla cuculus*	Triglidae	17
Red mullet	*Mullus barbatus*	Mullidae	3
Sardine	*Sardina pilchardus*	Clupeidae	2, 3, 5, 15
Spotted flounder	*Citharus linguatula*	Citharidae	12

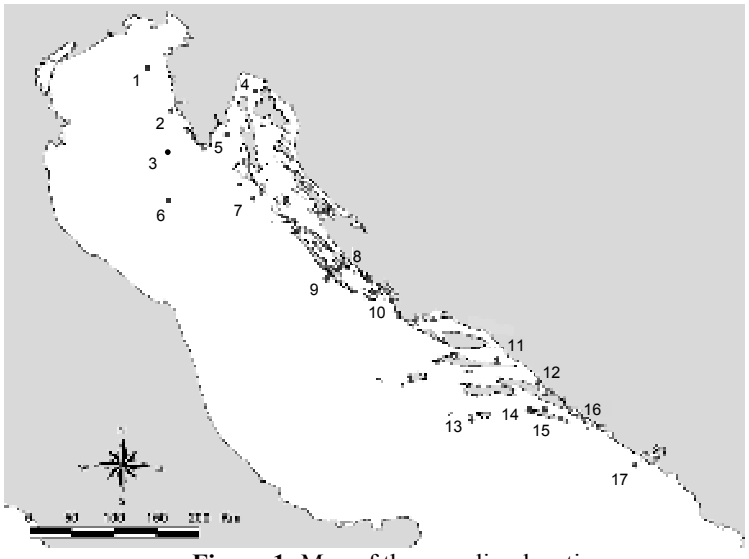

Figure 1: Map of the sampling locations

Results and discussion:

Table 2: OCPs concentrations (ng g^{-1} fat weight) determined in 34 samples of edible fish species collected in coastal and open waters of the Eastern Adriatic (Croatia)

Compound	Number of positive samples	Range	Median	Lower Quartile (25%)	Upper Quartile (75%)
HCB	4	<dl - 2.8	<dl	<dl	<dl
α-HCH	4	<dl - 1.9	<dl	<dl	<dl
β-HCH	15	<dl - 1823	<dl	<dl	10.3
γ-HCH	3	<dl - 529	<dl	<dl	<dl
DDE	34	14.2-541	54.6	27.6	98.3
DDT	22	<dl - 6949	9.5	<dl	46.7
DDD	18	<dl - 4592	2.7	1.04	5.6
DDE/DDT	22	0.09-8.8	3.2	<dl	9.1

dl – detection limit

γ-HCH , α-HCH and HCB were found in only few samples (Table 2). All found concentrations were lower than 3 ng g^{-1} fat weight except for one sample (location 15) where high concentration of γ-HCH was found (529 ng g^{-1} f.w.). β-HCH was found in 44 % of analysed samples, and the highest concentration was found also in sample collected in small harbour at island Mljet (location 15) where traffic of yachts and small boats is relatively intense. Elevated β-HCH concentrations were also found in samples from locations 3, 6 (both from North Adriatic), and 12 (located in river Neretva watershed, where local population is dominantly rural), although they were 2-10 fold lower then in sample from location 15.

DDE was the only organochlorine pesticide detected in all samples. Naso et al.[3] also found DDE to be the most frequently detected OCP in marine species. DDE is the main DDT metabolite characterised with high persistence in aquatic environment. Since the DDE/DDT ratio is often used as indicator of recent DDT inputs into the environment, the increase in the relative abundance of DDE within the DDT mixture (DDE/DDT >1 in 16 samples) shows the absence of new DDT inputs in the region due to the ban of its use. DDE/DDT ratios lower or close to 1 were found in samples collected in the open waters of the North Adriatic (locations 3 and 6), in the river Neretva watershed (location 12) and in small harbour at island Mljet (location 15). That implies fresh inputs of DDT at these locations, which agrees well with findings of Kljaković-Gašpić et al.[4] who investigated mussels from the Adriatic Sea. This is also in agreement with reported use of DDT in the Mediterranean region[5].

DDD was found in almost all samples containing DDT, with the highest concentration found in sample of anchovy from the location 3. The following, 16 fold lower concentration was found in sample of hake from location 12.

Croatia has no legislative regulation on maximum permissible levels (MPL) for OCPs in fish. Just for orientation, 4 samples (out of 34) exceeded prescribed MPL for OCPs in meat and meat products[6]. These are samples of anchovy (station 3) and hake (locations 6 and 12) in which levels of β-HCH and ΣDDTs (DDT+DDD+DDE) exceeded MPL, and sample from station 15 in which levels of β-HCH and γ-HCH were higher than MPL.

Discussion over OCP levels reveals that, out of 17 locations along the coast from which the samples were collected, significant OCP concentrations were found only in three areas: in open waters of the North Adriatic, in the Neretva river watershed and in small harbour at Mljet Island. Elevated levels of OCPs in fishes collected in river Neretva watershed are not very surprising. In this area local population is dominantly rural so there is possibility of illegal use of pesticides despite the ban of OCP use, and/or washout of soil and sediment contaminated by previous use. The area around Neretva is well-known breeding area of many agricultural products, including tangerine, watermelon, tomato, grapes etc. Outstandingly high β-HCH and γ-HCH levels in fish collected at sampling location in Mljet

Island are possibly the consequence of local point source in harbour. North Adriatic is influenced by strong fresh water inflow coming mainly from the Po River (North-west Adriatic, Italy), which is the longest river in Italy that drains one of the most populated and industrialized regions in Italy. Except for β-HCH and γ-HCH in above mentioned sample from the station 15, levels of HCB and HCH isomers found in this study do not stand out in comparison to the levels found by other authors[3,7].

In Southern Italy Naso et al.[3] found DDE in higher concentrations in comparison to DDD and DDT in all fish samples. For fish samples from Central Adriatic Sea Perugini et al.[8] reported concentrations following decreasing trend: DDE>DDD>DDT. Similar is with samples from the Marmara Sea, Turkey[7], although DDD was in some cases found in higher levels than DDE. By looking only the upper value of ranges for DDD and DDT, levels found in our study exceed by far the ones found in Turkey (44-287 ng g^{-1} f.w. for DDD; 17-183 ng g^{-1} f.w. for DDT), Central Adriatic Sea (<0.002-84 ng g^{-1} f.w. for DDD; <0.002-22 ng g^{-1} f.w. for DDT) and Southern Italy (<dl-265 ng g^{-1} f.w. for DDD; <dl-124 ng g^{-1} f.w. for DDT, dl=0.1-0.5 ng g^{-1}). But when comparing the medians for the same compounds, it is evident that our medians for DDD are much lower in comparison to other three locations, while DDT median is lower than for fish from Turkey, higher than for fish from Southern Italy, and very similar to the one found in Central Adriatic.

The upper value of the DDE range in our study is exceeding the one from Turkey (380 ng g^{-1} f.w.), but is close to the one from Central Adriatic (500 ng g^{-1} f.w.) and lower than the one for fish from Southern Italy (734 ng g^{-1} f.w.). By comparing the median values for DDE found in the same areas, three foreign studies have similar DDE medians that are 3 to 4 fold higher (171, 198 and 224 ng g-1 f.w. in Turkey, Central Adriatic and Southern Italy, respectively) than DDE median for this study. Bayarri et al.[8] found DDE in the range from 6.4 to 32 ng g^{-1} (median 9.8) in fish collected along Italian coast of the Adriatic Sea, but these values are expressed on fresh weight basis.

Although on first sight it seems like OCP concentrations found in fish from the Eastern Adriatic Sea are very high, all the values, except for few individual samples, are in agreement to previously reported values found in this region. This study also confirms that higher contamination levels are undoubtedly connected to areas with increased anthropogenic impact.

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