Bull. Environ. Contam. Toxicol. (2004) 73:331–338

DOI: 10.1007/s00128-004-0432-y

Bulletin of Environmental Contamination and Toxicology

Temporal Trend of Persistent Organic Pollutants in Codfish-Liver from the Adriatic Sea, Mediterranean Sea, 1993–2003

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Received: 22 January 2004/Accepted: 13 May 2004

Among the wide range of organic substances contaminating the aquatic environment a major concern has so far been focused on polychlorinated biphenyls and organochlorine pesticides. High persistence and biological degradation resistance of these toxic organic pollutants make them continue to be yet largely present in the marine environment although many countries have already and from long time restricted or withdrawn their use.

Aquatic organisms may bioconcentrate these environmental contaminants to more than 1000000 times the concentrations detected in the water column (US EPA 1992). Fish tissue monitoring serves as important indicator of environmental contamination and many States routinely conduct tissue analyses as a component of their comprehensive environmental monitoring programmes. The long-time monitoring studies are valuable tools of estimating the trends of environmental pollution by persistent chemicals and temporal trend data also help to predict the future impacts of toxic substances (Kannan et al. 1992). Codfish liver rich in fat, concentrating easily these lipophilic substances, constitutes an organ particularly suitable to be used in long-term monitoring of such contaminants (Falandysz 1981; De Boer, 1989; Kannan et al. 1992; Sinkkonen and Paasivirta 2000).

Adriatic Sea is an arm of the Mediterranean Sea separating Italy from the Balkan peninsula. The Adriatic extends northwest from 40° to 45° 45' N, with an extreme length of about 480 miles and a mean breadth of about 100 miles. This marine area connected by the Strait of Otranto, wide only 45 miles, with the Ionian Sea, for its nature of semiclosed basin, with scarce water reflux, constitutes an ecosystem at risk for accumulation of toxic compounds. It receives extensive urban and industrial waste water discharge from the bordering countries and it is also subjected to the intakes of great rivers. For this specific marine area to our knowledge it seems that there exists no comprehensive information on the historical trends of a wide variety of organochlorine compounds for long and continuous period of sampling years. In this context concentrations of polychlorinated biphenyls, organochlorine pesticides, hexachlorobenzene and hexachlorocyclohexane isomers were determined in codfish liver collected from the Adriatic Sea during 1993-2003 in order to estimate the long-term changes in residue levels and the present status of contamination of this aquatic zone.

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MATERIALS AND METHODS

Codfish (*Merluccius merluccius*) were caught in the Adriatic Sea during May-June 1993-2003 each three years. Since animal size is recognized to be of importance in determining the organochlorine compound burden, specimens of similar length (30 cm) were selected for each year. The livers of a large number of these codfish (100 specimens for each year) were carefully removed, pooled and analyzed. Detailed analytical procedures to determine chlorobiphenyls (PCBs 8, 20, 28, 35, 52, 60, 77, 101, 105, 118, 126, 138, 153, 156, 169, 180 and 209), DDT compounds (DDTs = p,p'-DDT, o,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDD), hexachlorobenzene (HCB) and hexachlorocyclohexane isomers (HCHs = αHCH, βHCH, γHCH) concentrations have already been published elsewhere (Storelli and Marcotrigiano, 2001, 2003). Briefly, aliquots (3 g) of the homogenised samples were ground with anhydrous sodium sulphate and the mixture was extracted with petroleum ether (Erney, 1983). The extracts were concentrated and subsamples were taken in order to determine the tissue fat content by gravimetry. An aliquot (about 200 mg) of the remaining extract was dissolved in hexane (5 ml) and mixed with H_2SO_4 conc. for the clean up (Murphy 1972). After centrifugation, the hexane solution was concentrated (about 1 ml) and transferred on a glass column (i.d. 5 mm) filled with 1 g of florisil (activated at 120°C for 16 h) for the separation of PCBs from other organochlorine compounds. Analyses were made on a Carlo Erba HR gas chromatograph 8000 Top with automatic injection system and with an electron capture detector ECD-400, Ni^{63}(temperature: 350 °C). For all the analyses a fused-silica capillary column DB-5 Supelco (length = 30 mt, inside diameter 0.25 mm and film thickness 0.25 μm), was used. The individual PCB congeners were determined against the corresponding individual standards obtained from ULTRA Scientific, Inc. (chemical purity 99%). The identity of the DDT group compounds was confirmed by an alkali conversion to their respective olefins and re-analysis by GLC. Analytical data, as for DDT group compounds were obtained by a comparison between sample peak area and external standards peaks area (POCs mixture, bought from Supelco). Recoveries were determined by adding known amounts of PCBs, DDTs HCB and HCHs standards (at three levels of concentrations) to empty samples before extraction (method of additions) The recoveries were within 80-110%. Residues in 100% of the samples were confirmed by gas-liquid chromatography-mass spectrometry (Fisons MD 800). Concentrations of PCBs, DDTs, HCB and HCHs are presented as ng/g on lipid weight basis.

RESULTS AND DISCUSSION

Residual concentrations of polychlorinated biphenyls, organochlorine compounds, hexachlorobenzene and hexachlorocyclohexans in cod-liver samples collected from 1993 to 2003 are presented in Table 1.

Table 1. Polychlorinated biphenyls, organochlorine compounds, hexachlorobenzene and hexachlorocyclohexanes (ng/g lipid weight basis) in codfish liver from the Adriatic Sea during 1993-2003.

Years	PCBs	DDTs	HCB	HCHs
1993	1380±2.1	1132±1.9	14.7±1.2	7.6±0.9
1996	1420±1.8	930±2.0	16.5±1.0	4.8±1.0
1999	1190±2.5	750±2.3	12.9±1.3	3.3±0.8
2003	943±2.3	618±2.1	8.6±1.1	3.7±0.8

mean of 3 replicates

Polychlorinated biphenyls were the dominating pollutants among the organochlorine residues determined in the livers of cod and were found in all samples in each year considered. Highest polychlorinated biphenyl values were found in cod-liver samples of 1996 (1420 ng/g lipid weight basis), though the concentrations relative to samples caught in the next years (943-1190 ng/g lipid weight basis) indicated a slight, but nonsignificant, reduction in PCB levels. Data in literature, reporting PCB concentrations in different aquatic ecosystems, showed a rapid and substantial decline of these contaminant concentrations in the decade immediately next to introduction of regulatory restrictions in the use of these compounds (Skare et al. 1985; Olsson and Reutergardh 1986; De Boer 1989). Today at almost thirty years from the first restriction on PCB usage in Europe, adopted in 1976, it is apparent that they yet persist and in some marine areas continue to exhibit high residue levels (Environmental Agency Japan 1995). In the present the lack of a strong temporal decline of PCB contamination could be the result of a continuous and constant input of these pollutants in the area investigated. On the other hand if it were not so a drastic reduction in the concentrations of these contaminants in a decade should have been observed because their residence time in waters is estimated at 2-4 years (Burns et al. 1985). The results of a recent work (Frignani et al. 2001) regarding the presence of PCBs in sediments of the inner part of the Adriatic Sea (Venice Lagoon) confirm the presence of a variety of sources discharging PCBs.

Isomer-specific analysis of PCBs revealed similarities in cod-liver samples from different years. The hexachlorobiphenyls were the most abundant, followed by penta-, hepta-, and by tetrachlorobiphenyls, while trichlorobiphenyls were absent (Fig. 1). Also the contents of individual PCBs were rather similar in different years. Hexachlorobiphenyls 153 and 138, as well as heptachlorobiphenyl 180 were the predominant congeners together accounting from 58 to 65% of the total PCB concentrations. Other chlorobiphenyls found in relatively high percentage were PCB 118 (9.5-17.5%) and PCB 101 (7.8-13.4%), while the other congeners contributed to total PCB with very low percentages. This PCB congener profile pattern is in accordance with observations from several other study in cod from Scotland (Kelly and Campbell 1994), Baltic (Atuma et al. 1996), north Atlantic (Stange et al. 1996) and Norway (Sinkkonen and Paasivirita 2000; Green and Knutzen 2003).

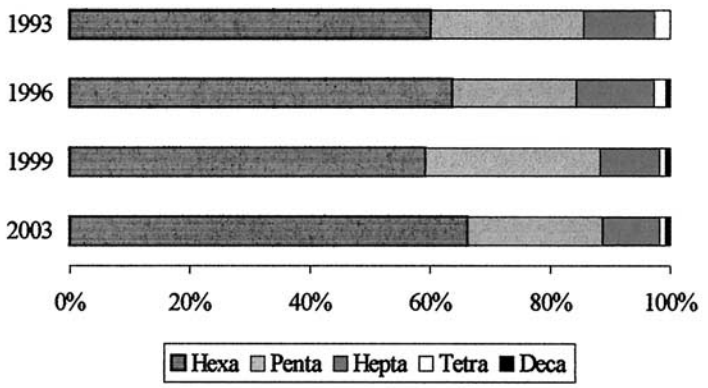

Figure 1. Chlorine- based classes of PCBs measured in cod-liver from the Adriatic Sea during 1993-2003.

The prominence of these highly chlorinated congeners was also observed in other marine organisms from Adriatic Sea, i.e. cartilaginous fish, (Storelli and Marcotrigiano 2001), dolphins (Kannan et al. 1993; Storelli and Marcotrigiano 2003) as well as marine turtles (Storelli and Marcotrigiano, 2000) indicating a predominant exposure to higher chlorinated PCB formulation. On the other hand, also sediments from Adriatic Sea contained a high proportion of more chlorinated PCBs with the pattern resembling Aroclor 1260 formulation (Galassi et al. 1993; Frignani et al. 2001), one of the most largely used commercial mixtures in European countries in the past, but still commonly used as a reference standard for biological samples of these areas.

In contrast to polychlorinated biphenyls, DDTs showed a substantial reduction over a period of 10 years running from a maximum value of 1132 ng g^{-1} lipid weight basis in 1993 to a minimum of 618 ng g^{-1} lipid weight basis in 2003. In general, DDTs composition in cod-liver showed, that p,p'-DDE had the highest influence (62-80%) on the total DDT burden, followed by p,p'-DDT (10-21%), p,p'-DDD (5-11.7%), o,p'-DDT (1-3.2%) and o,p'-DDD (0.9-4.7%) (Fig. 2). The decline in concentrations of these contaminants in the marine environment can be ascribed to the restrictions against the use of p,p'-DDT introduced in the beginning of the 1970s in different countries of Europe.

For example, Swedish ban on DDT came in 1969, while in Switzerland and Poland prohibition on the general use of DDT was imposed in 1972 and 1974, respectively. In Italy the prohibition on the use of DDT was later, coming only in 1978 (G.U., 1978).

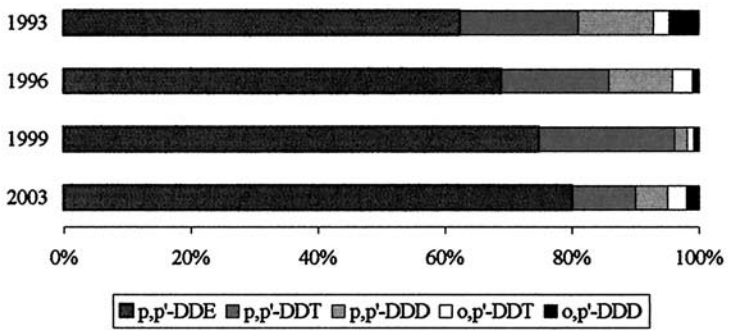

Figure 2. Percentage composition of DDT compounds in cod-liver from the Adriatic Sea during 1993-2003.

This declining tendency of DDT following ban the was observed in many countries by several authors (Andersson et al. 1988; Kannan et al. 1992; Bignert et al. 1998). It has been generally assumed that ratio of p,p'-DDE to total DDT constitutes an indicator of whether new sources of DDT are entering an ecosystem (Aguilar 1984). Values greater of 0.6 have been associated with old inputs of pollutants in the ecosystem. In our case, such ratio values (1993: p,p'-DDE/DDTs =0.63; 1996: p,p'-DDE/DDTs=0.69; 1999: p,p'-DDE/DDTs= 0.75; 2003: p,p'-DDE/DDTs =0.80) above this threshold, particularly from 1996 on, indicated no new inputs of DDT into the ecosystem.

Time trend of hexachlorobenzene concentrations showed a steady state in the period from 1993 to 1999 (12.9-16.5 ng g^{-1} lipid weight basis) followed by a slight decline observable in 2003. This compound has been used for various applications ranging from an active ingredient in fungicides to the production of chlorinated chemicals and it is also a byproduct of incineration of municipal waste. Though usage of HCB as fungicide in agriculture has been banned in several countries, officially reported data from Europe relatively to 1990s, showed that agricultural application was still the major source of HCB release. However, the overall amount of HCB releases in 1998 was lower by a factor of 8 compared to the values in the 1970s (Shatalov et al. 2001). In our study the slow rate of HCB reduction during the ten years period is in accordance with what reported in a extensive study of temporal trends of organochlorine in Northern Europe from 1987-1998 (Sinkkonen and Paasivirta 2000). Reemission due to volatilization of HCB from the soil contaminated from past use may be a plausible reason of unchanged concentrations of this compounds in the environment.

Hexachlorocyclohexanes were in concentrations much lower than those of the other contaminant. In this respect it is well to remember that HCH isomers are relatively biodegradable and less lipophilic compared to the other organochlorine

compounds showing thus low biomagnification factors in aquatic ecosystems (Colombo et al. 1995). With regard to hexachlorocyclohexane isomer composition, αHCH was prevalent (55-60%), followed by βHCH, while γHCH present in modest amount in samples of 1993 and1996 was absent in the others.

The low concentrations of these compounds are in agreement with the expected global decrease of HCHs concentrations after the ban or restriction in their usage in some countries. However, it is worthy to underline that some recent investigations have suggested a large usage of HCHs in Russia (Nakata et al. 1998), as well as in China and in India (Li et al. 1996). The possibility of long-range atmospheric transport, made easier by the high atmospheric mobility of these substances (Iwata et al. 1993; Wania and Mackay 1996) from tropical regions where they are still used emphasized the importance of HCHs as global pollutants.

It has been elucidated from our results that concentrations of different organochlorine compounds revealed a decrease more or less marked during the ten years period investigated. This is very reassuring, particularly because true on the global scale, since similar trends are also discernible from published reports from other parts of the world. However, despite these encouraging declines, contamination of the marine area investigated by the above pollutants it is not to understimate. Semi-enclosed seas are, in fact, among the areas deserving particular attention for future investigations pertaining to persistent organochlorine pollutant dynamics and their impact on the environment and on humans. Therefore, periodic monitoring of such persistent compounds with a special emphasis to PCBs should be instituted so as to get information on the environmental quality of these waters.

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