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Assessment of several priority pollutants in fish from selected lakes in Bulgaria

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The aim of the study was to assess the human health risk through fish consumption due to persistent organic pollutants like polychlorinated biphenyls (PCBs), DDT and its metabolites, hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD). The present study evaluates the human daily intake of priority pollutants through consumption of freshwater fish from some lakes in Bulgaria (Varna Lake, Beloslav Lake, Burgas Lake, Mandra Lake). Concentrations of organochlorine compounds were determined in six fish species: gibel carp *(Carassius gibelio)*, roach *(Rutilus rutilus)*, perch *(Perca fluviatilis)*, goby *(Neogobius melanostomus)*, golden grey mullet *(Mugil auratus)* and silverside *(Atherina boyeri)*.

The PCBs, HCB, HCBD, DDT and its metabolites DDE and DDD were determined by gas chromatography system with mass spectrometry detection. The sum of DDTs was determined from 1.81±0.16 to 11.31±1.26 ng/g wet weight (in perch and golden grey mullet, respectively). The other contaminants HCB and HCBD were found below the analytical detection limit. The sum of Indicator PCBs ranged from 1.00 (goby) to 5.30 ng/g ww (golden grey mullet).

The EDI of DDTs in fish from coastal lakes was calculated between 0.34 and 2.13 ng/kg body weight/ day through consumption of perch and golden grey mullet, respectively. EDI of I-PCBs in fish was between 0.19 and 1.00 ng/kg bw day through consumption of goby and golden grey mullet, respectively. The health risks were assessed using a risk quotient (RQ) of the fish consumption as the ratio of daily fish exposure level in relation to oral reference dose. All the RQ values were much lower than 1, suggesting that consumption of the fish species from coastal lakes in Bulgaria would not pose a non-cancer risk for humans.

Keywords: *PCBs; DDTs, fish; risk assessment; Bulgaria*

INTRODUCTION

Persistent organic pollutants (POPs) are a group of compounds, which are characterised by their ability to persist in ecosystems, their high lipid solubility and their bio-magnification in the food chain [1]. POPs accumulate in the fatty tissue of living organisms, reaching the greatest concentrations at the top of the food chain in fish, mammals and predatory birds [2]. Polychlorinated biphenyls (PCBs) and 1,1,1-trichloro - 2, 2 - bis (4-chlorophenyl) ethane (DDT) and its metabolites (DDTs) are highly lipophilic compounds and they rapidly accumulated in living organisms [3]. Although the usage for agriculture of DDTs has been banned since 1970s, DDTs are still being used in low amounts to control certain insects in tropical and subtropical countries [4]. Hexachlorobenzene (HCB) is a hydrophobic and highly persistent compound [5]. Although hexachlorobenzene is not currently manufactured, it is formed as a waste product in the production of several chlorinated hydrocarbons and is a contaminant in some pesticides [6]. The main source of HCB today is chemical industry from which this compound can be emitted as a product in high-temperature processes [7]. Hexachlorobutadiene (HCBD) was mainly used as an intermediate in the manufacture of rubber compounds and other polymers. Other uses were in agriculture as a seed dressing, in hydraulic fluids and a number of other industrial processes [8]. HCB and HCBD are also named as priority substances under the EU Water Framework Directive [9].

These very persistent pollutants have the potential to affect the physiological functions of wildlife [10]. Although humans can be exposed to POPs through direct exposure, occupational accidents and the environment, most of the human exposure nowadays is from the ingestion of contaminated food as a result of bioaccumulation in the food chain [11, 12]. It has been reported that meat, dairy products and fish, makes up more than 90% of the intake of POPs for the general population [13, 14, 15]. Data on the presence and distribution of organohalogenated contaminants in fish and especially edible fish species are important not only from ecological, but also from human health perspective [16].

There are several lakes along Bulgarian Black Sea coast. Varna Lake is the largest by volume and deepest lake along the Bulgarian Black Sea Coast, and having an area of 17 km² and a volume of

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166 million m³. A number of rivers pour into the lake near the western shores of Beloslav Lake, which is connected to Varna Lake. Burgas Lake is located near the Black Sea, west of the Burgas city, is the largest natural lake in Bulgaria, with an area of 27.60 km². An important fish-producing reservoir in the past, Burgas Lake lost much of its economic importance after the construction of the petrochemical plant near the city, but has witnessed an increasing number of species and decreasing pollution in recent years. Mandra Lake is the southernmost of the Burgas Lakes, located in the immediate proximity of the Black Sea. Parts of Mandra Lake are designated protected areas inhabited by a number of locally and globally endangered species of fish and birds.

A lot of local fishermen consume fish from these coastal lakes. Therefore, it is very important to clarify the status of POPs in fish from these waters and the present study will provide more information on the residues of persistent organic pollutants in fish from Varna Lake, Beloslav Lake, Burgas Lake and Mandra Lake.

The aims of the present study were to evaluate the daily intake and to assess the human health risk of priority pollutants (PCBs, DDTs, HCB and HCBD) through consumption of fish from some coastal lakes in Bulgaria.

EXPERIMENTAL

Sampling

Six wild fish species: gibel carp *(Carassius gibelio)*, roach *(Rutilus rutilus)* and perch *(Perca fluviatilis)*, goby *(Neogobius melanostomus)*, golden grey mullet *(Mugil auratus)* and silverside *(Atherina boyeri)* were sampled from some coastal lakes in Bulgaria (Varna Lake, Burgas Lake, Burgas Lake and the Mandra Lake). Samples were caught by local professional fishermen between September and November 2014. Samples were immediately transferred to the laboratory in foam boxes filled with ice and were stored in a freezer (-18ºC) until analysis.

Analytical method

The method used for the preparation of the samples, clean-up and quantitative determinations of PCBs in fish samples has been previously described in details [17]. The edible tissue of each fish was homogenized using a blender; pools of about 300 g were made with fillets taken from several individual fish. Briefly, twenty grams of homogenized fish tissue were extracted with hexane / dichloromethane in Soxhlet Extractor. The extract was cleaned-up on a glass column packed with 2 g neutral silica, 4 g acid silica and 2 g neutral silica (Merck KGaA, Darmstadt, Germany). The eluates were concentrated to near dryness and reconstituted in 0.5 ml in hexane. One micro liter of purified extract was injected into GC/MS.

Gas chromatographic analyses of PCBs were carried out by GC FOCUS (Thermo Electron Corporation, Austin, Texas, USA) using POLARIS Q Ion Trap mass spectrometer. Splitless injections of 1 µl were performed using a TR-5MS capillary column (Bellefonte, PA, USA) coated with cross-linked 5% phenyl methyl siloxane with a length of 30 m, 0.25 mm ID and a film thickness of 0.25 µm. Helium was applied as carrier gas at a flow of 1 ml/min.

Pure reference standard solutions (PCB Mix 20 - Dr. Ehrenstorfer Laboratory, Augsburg, Germany), were used for instrument calibration, recovery determination and quantification of compounds. Measured compounds were: the six Indicator PCBs (I-PCBs IUPAC No. 28, 52, 101, 138, 153 and 180) and six dioxin-like PCBs (non-ortho PCBs 77, 126, 169 and mono-ortho PCBs 105, 118, 156). Each sample was analyzed three times and was taken an average of the results obtained.

Quality control

The quality control was performed by regular analysis of procedural blanks and certified reference material BCR - 598 (DDTs in Cod liver oil) and BB350 (PCBs in Fish oil) – Institute for Reference Materials and Measurements, European Commission. Recovery of PCBs from certified reference material varied in the range 85 -109% for individual congeners. Procedural blanks and a spiked sample with standards were analyzed between each 5 samples to monitor possible laboratory contamination. Blanks did not contain traces of contaminants.

Statistical analysis

The statistical analysis of the data was based on the comparison of average values by a t-test and a significance level of $p<0.05$ was used. When the p value was lower than 0.05, it was considered statistically significant. All statistical tests were performed using SPSS 16 software. For the purpose of statistical analysis, concentrations of contaminants reported as "not detected" were assigned as the detection limit. The data used in the present study were based on the mean concentrations of the target contaminants in the fish species.

Dietary intake estimation

Human exposure assessment of POPs through oral ingestion is generally estimated using daily intake of the contaminant. The estimated total daily intake (EDI) of the contaminants in a given fish species was calculated as follow [18]:

$$EDI = C \times Intake / BW$$

where EDI is the estimated daily intake (ng/kg body wt./day), C is the average concentration of measured POPs (ng/g wet weight), Intake is the daily food consumption of fish (13.2 g/ day for Bulgarian standard adult [19] and BW is the average consumer body weight (70 kg for adult men).

Risk assessment

Risk assessments were conducted based on the concentrations of organochlorine pesticides and PCBs compounds in fish tissues. The potential risks of non-carcinogenic effects are evaluated by the risk assessment index known as the risk quotient (RQ). RQ is defined as the ratio of daily fish exposure level (EDI) in relation to reference dose (RfD) considering non-carcinogenic effects of the contaminants. The RQ was calculated as follows [18, 20]:

$$RQ = EDI / RfD$$

where EDI is the estimated daily intake (ng/kg body wt./day); and RfD is the reference dose (ng/kg day). RfD values adopted in this study are the criteria of the USEPA (Environment Agency of the United States) [20].

RESULTS AND DISCUSSION

Indicator PCBs levels

PCBs and chlorinated pesticides have been monitored routinely in the environment and foodstuff in various countries to evaluate their potential health risk to humans [21, 22]. Consumption of contaminated food is an important route of human exposure to organochlorine compounds. The sum of the six PCBs (IUPAC № 28, 52, 101, 138, 153 and 180) comprises about half of the amount of total non dioxin-like PCBs present in feed and food [23]. They are called indicator PCBs (I-PCBs) for evaluating the risk to human health [24]. The concentration levels of individual PCBs congeners in fish from coastal lakes along Bulgarian Black Sea coast were described in our previous studies [25, 26]. Our previous studies showed that the most abundant PCB congeners in fish species were the indicator PCBs constituting more than 80% of the total amount of PCBs [25, 26].

The lipid content, mean levels of Total Indicator PCB congeners in investigated fish species from coastal lakes in Bulgaria, estimated daily intake (EDI) and risk quotient (RQ) are shown in Table 1. The lipid percentage ranged from 0.5% (goby) to 6.1% (silverside).

The mean levels of I-PCBs ranged between 1.00 ng/g ww (goby) and 5.30 ng/g ww (golden grey mullet), calculated as the sum of 6 Indicator PCB congeners. The differences in concentrations of PCBs may be attributable to various factors such as the nature of the habitat, feeding preferences and lipid contents. The higher levels of PCBs in grey mullet compared to other fish species may be due to its nature of the habitat. These species usually inhabit muddy bottoms along the coast, and ports and estuaries, which are generally considered to be more heavily polluted than open waters. Golden grey mullet probably receive large quantities of organochlorine pollutants present in the water and in the sediments through a process of bioconcentration [27]. The European Union has recommended a maximum level of 75 ng/g wet weight, calculated as the sum of the six I-PCBs in muscle meat of fish [24]. Our results for Sum of I-PCBs in all fish species did not exceed this limit.

The pattern of indicator PCBs found in wild fish from coastal lakes showed a predominance of PCB 153 (31.9%) followed by PCB 101 (24.3%) for indicator PCBs (Table 2).

The predominance of hexachlorinated and pentachlorinated PCBs in fish species, especially PCB 153, PCB 101 and PCB 138, has been reported by several authors for different coastal areas in the Mediterranean [27] and in the Adriatic Sea [28]. The distribution of PCB congeners could be explained by the fact that that the accumulative properties of PCB congeners increase with the number of chlorine atoms substituted to the hydrogen atoms in biphenyl rings and the resulting increase in their lipophilicity [29].

Estimated daily intake (EDI) of I-PCBs

Fish and seafood accounts for a small portion of human diet, but it has been proven to be one of the major routes of human exposure to organic contaminants [12]. The consumption of contaminated fat food can be a potential risk for the consumer. To comprehensively evaluate risk exposure, the mean EDIs for these harmful chemicals in each fish species were calculated. On the basis of the measured concentrations in the fish samples, the daily dietary intake of PCBs was

Table 1 Lipid content (%), levels of Total Indicator PCBs (ng/g wet weight, mean and standard deviation) determined in fish from coastal lakes in Bulgaria, Estimated daily intake (EDI) and Risk quotient (RQ).

Species	n	**Lipids**, %	**Sum I-PCBs,** *ng/g ww*	**EDI,** *ng/kg bw day*	**RfD,** *ng/kg/day* (USEPA)	**RQ**
gibel carp	8	1.3±0.4	1.60±0.37	0.30±0.08	20	0.015
roach	6	2.2±0.2	1.06±0.25	0.20±0.04	20	0.010
perch	8	0.6±0.1	1.06±0.22	0.20±0.04	20	0.010
goby	8	0.5±0.1	1.00±0.16	0.19±0.03	20	0.009
golden grey mullet	6	4.2±1.2	5.30±0.56	1.00±0.13	20	0.050
silverside	10	6.1±1.6	3.99±0.34	0.75±0.14	20	0.038

RfD – oral reference dose

Table 2 The PCB pattern (% of total indicator PCBs), estimated daily intakes of individual I-PCBs in fish (mean value) for adults (aged 15–75) in ng/ kg body weight per day.

Substance	**% of total I-PCBs**	**Mean concentration,** *ng/g ww*	**EDI,** *ng/kg bw day*	**RfD,** *ng/kg/day* (USEPA)
PCB 28	17.1	0.42±0.03	0.08	20
PCB 52	17.8	0.43±0.04	0.08	20
PCB 101	24.3	0.59±0.06	0.11	20
PCB 138	4.9	0.12±0.02	0.15	20
PCB 153	31.9	0.77±0.06	0.02	20
PCB 180	3.9	0.10±0.02	0.02	20

Table 3 Levels of Total DDTs (ng/g wet weight, mean and standard deviation) determined in fish collected from coastal lakes in Bulgaria, Estimated daily intake (EDI) and Risk quotient (RQ).

Species	n	**Sum DDTs,** *ng/g ww*	**EDI,** *ng/kg bw / day*	**RfD,** *ng/kg/day* (USEPA)	**RQ**
gibel carp	8	3.99±0.37	0.75±0.07	500	0.002
roach	6	2.45±0.25	0.46±0.06	500	0.001
perch	8	1.81±0.16	0.34±0.03	500	0.001
goby	8	2.69±0.26	0.51±0.05	500	0.001
golden grey mullet	6	11.31±1.26	2.13±0.23	500	0.004
silverside	10	6.79±0.64	1.28±0.13	500	0.003

calculated. The estimated daily intake of the Sum I-PCBs in fish species studied are shown in Table 1. The EDI was calculated on the basis of a fish consumption rate of 13.2 g/day [19] for adults with body weight of 70 kg, on the mean exposure level. The EDI of I-PCBs in fish from coastal lakes was calculated between 0.19 and 1.00 ng/kg bw day through consumption of goby and golden grey mullet, respectively and was far below recommended RfD of 20 ng/kg bw day for adults [20]. Overall, the EDIs of these POPs via fish consumption for adults in the present study were lower than those reported in most previous studies [23, 30].

Levels and estimated daily intake (EDI) of DDTs

Because of their wide distribution in air, water, soil and food, p,p'-DDT and its metabolites (p,p'-DDD and p,p'-DDE) remain a human health concern and have been determined in edible fish tissues from investigated coastal lakes in Bulgaria [25, 26]. Summarized data of mean levels of total DDTs (like sum of p,p'-DDT, p,p'- DDD, p,p'-DDE) found in the fish samples and estimated daily intakes are present in Table 3.

The maximum level of Sum DDTs was found in golden grey mullet (11.31 ng/g ww), while the minimum value was found in perch (1.81 ng/g ww). The experimental results showed significant differences of DDTs levels between different fish species (statistical test – $p<0.05$). The daily intake of DDTs (like sum of p,p’-DDT, p,p’- DDD, p,p’-DDE) was calculated on the basis of the measured concentration in fish species (Table 3). The mean EDI of total DDTs in fish from Varna Lake, Lake Burgas and Mandra Lake was calculated between 0.34±0.03 and 2.13±0.19 ng/kg body weight/ day through consumption of perch and golden grey mullet, respectively. The US Environmental Protection Agency established a Reference Dose (RfD) of 500 ng/kg body weight day [20], which corresponds to a tolerable daily intake of 0.5 μg/kg body weight from Integrated Risk Information System (IRIS) [31] for the non-carcinogenic effects. The mean EDI of 2.13 ng/kg body weight per day for adults is well below this value.

The distributions of levels PCBs, DDTs, HCB and HCBD in wild fish from Varna Lake, Beloslav Lake, Burgas Lake and Mandra Lake are summarised in Table 4.

The comparison of DDTs, PCBs, HCB and HCBD residues detected in fish collected from different coastal lakes shows that DDTs concentration are significantly higher ($p<0.05$) in Varna Lake (10.32 ng/g ww) than those in Mandra Lake (2.20 ng/g ww). This is probably due to the influence of salty sea water in Varna Lake flowing from the Black Sea. Our previous studies have shown higher levels of organochlorine contaminants in marine fish species compared to freshwater species [32]. In term of PCBs, HCB and HCBD, no significant differences were detected between these four geographic locations.

In relation to other organochlorine compounds determined concentrations of HCB and HCBD were all below detectable levels (Table 4) and did not exceed the European EQS of 10 μg/kg and 55 μg/kg (in biota), respectively. HCB is known as volatile and practically insoluble in water compound which leads to a low bioavailability of this contaminant in marine organisms. In a recent study of wild fish from four English rivers HCB was a maximum of 6 μg/kg in some eels [8]. In a survey of eels in Scotland [16] HCBD was only detected in one of 150 samples at detection limits of either 1 or 3 μg/kg and the authors of a French study also failed to detect any HCBD in fish [33]. The concentrations of HCB and HCBD were found below detection limit in all fish samples and daily intake was not estimated for these two chemicals.

Human health risk assessment

Many authors have revealed that high fish and seafood consumption increases the risk of POPs contamination of the human body [3, 34]. Current non-cancer risk assessment methods are usually based on the use of the Risk quotient (RQ). RQ is a ratio between the estimated dose of a contaminant and the reference dose (RfD) below which there will not be any appreciable risk [20]. The RfD is an estimate of daily exposure in humans that is likely to be without an appreciable risk of deleterious effects during a lifetime [18]. The average consumption together with the measured concentration of the contaminant are used to calculate the risk quotient RQ. RfD values adopted in this study are the criteria of the USEPA (Environment Agency of the United States) [20]. If the RQ value is less than 1, no obvious health risks due to the intake or uptake of contaminants via fish consumption would be experienced. Conversely, an exposed population of concern will experience health risks if the fish consumption rate is equal to or greater than the RfD value [18].

Table 4 Comparison of mean organochlorine levels in fish from different coastal lakes in Bulgaria (concentration, ng/g ww).

Compound	Varna Lake	Beloslav Lake	Burgas Lake	Mandra Lake
	goby, golden grey mullet	goby, golden grey mullet, silverside	gibel carp, roach	roach, perch
PCBs	4.24±2.01	2.71±1.21	1.29±0.44	1.10±0.70
DDTs	10.32±4.25	4.72±2.04	3.15±1.19	2.20±0.55
HCB	<LOD	<LOD	<LOD	<LOD
HCBD	<LOD	<LOD	<LOD	<LOD

<LOD – below limit of detection

Regarding risk assessment due to PCBs, the RQ values ranged from 0.009 to 0.050 for goby and golden grey mullet, respectively (table 2). The RQ values for DDTs in fish samples (presented in Table 3) were calculated from 0.001 (roach, perch, goby) to 0.004 (golden grey mullet). All the RQ values were much lower than 1, suggesting that consumption of the fish species would not pose a non-cancer risk.

CONCLUSION

The indicator PCB levels found in fish species from coastal lakes along Black Sea coast ranged between 1.0 ng/g ww (goby) and 5.3 ng/g ww (golden grey mullet) and did not exceed the maximum EU limit of 75 ng/g ww. The lower observed levels of PCB in fish tissues than from fish tissues of other aquatic ecosystems was potentially due to the absence of PCB manufacturing in Bulgaria. The maximum level of Sum DDTs was found in golden grey mullet (11.31 ng/g ww), while the minimum value was found in perch (1.81 ng/g ww). The estimated daily intakes of Indicator PCBs and DDTs by humans were far below RfD or the TDI for adults, recommended by US EPA and FAO/WHO, indicating that this intake would not pose a health risk. Human health risk assessment, based on RQ values much lower than one, suggesting that consumption of the fish species from coastal lakes in Bulgaria would not pose a non-cancer risk.

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ОЦЕНКА НА НЯКОИ ПРИОРИТЕТНИ ЗАМЪРСИТЕЛИ В РИБИ ОТ ИЗБРАНИ ЕЗЕРА В БЪЛГАРИЯ

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(Резюме)

Целта на настоящото изследване беше да се оцени здравния риск чрез консумацията на риба по отношение на устойчиви органични замърсители като порихлорирани бифенили (ПХБ), ДДТ и метаболити, хексахлоробензен (ХХБ) и хексахлоробутадиен (ХХБД). Изчислен е дневен прием на приоритетните замърсители чрез консумация на сладководни риби от някои езера в България (Варненско езеро, Белославско езеро, Бургаско езеро и езеро Мандра). Концентрациите на органохлорните съединения бяха определени в шест рибни вида: каракуда *(Carassius gibelio)*, бабушка *(Rutilus rutilus)*, костур *(Perca fluviatilis)*, кая *(Neogobius melanostomus)*, платерина *(Mugil auratus)* и атерина *(Atherina boyeri)*.

ПХБ, хексахлоробензен, хексахлоробутадиен, ДДТ и основните му метаболити ДДЕ и ДДД са определени чрез газова хроматография с масспектрометричен детектор. Средният дневен прием на замърсителите в риби от крайбрежните езера са изчислени между 0.34±0.03 и 2.13±0.19 (за ДДТ и метаболити) и между 0.19±0.02 и 0.75±0.08 ng/kg телесно тегло дневно (за ПХБ) чрез консумация съответно на кая и платерина. Здравният риск беше оценен чрез използване на коефициент на риск (RQ) като отношение на дневната експозиция и оралната референтна доза (RfD). Изчислените RQ са под единица, което означава, че консумацията на риба не представлява риск за човешкото здраве.

Ключови думи: *ПХБ; ДДТ; риба оценка на риск; България*