LEVELS AND ENANTIOMERIC SIGNATURES OF α -HCH IN AN AQUATIC FOOD CHAIN FROM THE DANUBE DELTA, ROMANIA

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Introduction

Due to its high biodiversity, the Danube Delta is the largest wetland from Europe (580,000 ha), situated in the S-E of Romania and at N-W of the Black Sea. Considered the best preserved of Europe's deltas, the Danube delta hosts over 300 species of birds as well as 45 freshwater fish species in its numerous lakes and marshes[1]. Little is known about the degree of contamination with organohalogenated pollutants of the lower Danube River and Danube Delta. Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were measured in bird's eggs collected in 1997 from the Danube Delta[2] and it was found that DDTs are the main contaminants in all samples and that PCB concentrations are not as low as it might have been thought. In another study, high concentrations of PCBs and OCPs associated with sediments from the Danube indicated that the river is a major source of contamination to the Black Sea[3].

Although the widespread use of lindane and technical-grade hexachlorocyclohexane (HCH) has been discontinued in most countries for a long time, these residues continue to persist in the environment. Recently, it has been shown that organochlorine pesticides, including HCHs, are the main organochlorine pollutants in the Romanian environment[4]. HCHs are still used in Romania in several applications and their presence in the environment is of high concern, especially for the aquatic ecosystems[5]. α-HCH, the major component in technical HCH (approx 60%) is the only chiral HCH isomer. Recently, the importance of determining the enantiomeric levels and ratios of persistent pollutants in environmental samples has been emphasized[6]. Enantiomers may exhibit different biological and toxicological activity[7] and may also be helpful to assess the extent of enantioselectivity in the environment and the ability of biological systems to transform xenobiotic compounds. Enantioselective analyses may prove useful in characterizing other biological processes, such as the health status and food quality associated with a captive or wild population.

This study aims to evaluate the occurrence and distribution of enantiomers of α-HCH in biota (invertebrates, different species of fish and cormorant tissues) collected from the Danube Delta.

Methods

Samples. Sediments, zooplankton and chironomids (*Chironomus plumosus*) were collected from 3 different locations (Merhei, Rosu and Razim). Muscle was collected from 36 individual fishes of variable age (2-7 yrs), both females and males, with whole body weights ranging from 90 to 1300g caught in 3 different locations (Matita, Caraorman and Enisala). They have different feeding type: B- benthivorous (tench and bream), H- herbivorous (gibel carp), O- omnivorous (rudd, carp, white bream and roach) and P- piscivorous (wels, pikeperch, pike and perch). Liver and muscle tissues were collected from 4 common cormorants (*Phalacrocorax carbo)* caught in 2 locations (canal Papadia and Japca Marcova). All samples were collected between August and September 2001.
Analysis. All samples were previously analyzed for their concentrations of organochlorine pesticides (including α-, β- and γ-HCH), 18 PCB congeners and 7 PBDEs congeners[8].

For enantioselective analysis of α-HCH, available extracts were fractionated on a silica SPE cartridge. The 1st fraction, containing all PCBs, *p,p'*-DDE and *p,p'*-DDT, was eluted with 4 ml hexane, and the 2nd fraction containing all HCH isomers and *p,p'*-DDD, was eluted with 3 ml DCM. After concentration, the 2nd fraction was analysed by GC/ECNI-MS using a 30m x 0.25mm Chirasil-Dex column (Chrompack). A volume of 5 x 5 μl of the extract was injected in large volume injection mode. Three ions (71, 253 and 255) were monitored for α-HCH. The enantiomeric ratio (ER) was defined as the ratio of peak area of the first ((+) α-HCH) to the second eluting enantiomer ((-) α-HCH). A good reproducibility (RSD<1.5%) for injections of standard solutions allowed the determination with sufficient significance of ER changes of even a few percentages. The enantiomeric fraction (EF) was defined as ER/(ER+1).

Results and discussion

Mean concentrations of HCHs expressed in ng/g wet weight (ww) and EFs measured in different fish species and cormorant from Danube Delta are shown in Table 1. The sum of HCH concentrations was < 3 ng/g and < 20 ng/g ww for all studied fish species and cormorant tissues, respectively (Table 1). Concentrations of HCH isomers in fish samples varied between species and per location and they were closely related to feed intake and pattern. No clear trend in the concentrations of all HCH isomers could be observed for benthic to piscivorous species. Their low lipid percentage (between 0.2 and 2.0%) led to relatively high values of concentrations expressed per lipid weight (lw). Mean values ranged between 81 ng/g lw for pike and 250 ng/lw for bream, with two high maximum values of 360 ng/g lw and 375 ng/g lw for gibel carp and bream, respectively. For invertebrates (zooplankton and chironomids) which serve as food for many species of fishes, maximal values were 83 ng/g dry weight for HCHs (Rosu lake). Higher concentrations of HCHs were measured in cormorant samples from japsa Marcova than from canal Papadia, with β-HCH being the dominant HCH isomer in muscle (M) and liver (L).

Table 1. Mean values of HCH isomers concentrations (ng/g wet weight), and EFs for α-HCH for fish species and cormorant from the Danube Delta.

	N	EF	Concentrations (ng/g ww)			
			α-HCH	β-HCH	γ-HCH	ΣHCHs
Tench (*Tinca tinca*)	4	0.473	0.26	0.21	0.27	0.75
Bream (*Abramic brama*)	4	0.498	0.96	0.49	1.08	2.53
Gibel carp (*Carasius auratus gibelius*)	6	0.464	0.49	0.35	0.72	1.56
Rudd (*Scardinius erythrophtalmus*)	3	0.490	0.95	0.37	1.33	2.65
Carp (*Cyprinus carpio*)	2	0.465	0.71	0.36	0.46	1.53
White bream (*Blicca bjorkena*)	2	0.487	0.57	0.22	1.08	1.87
Roach (*Rutilus rutilus*)	2	0.495	0.67	0.51	1.55	2.73
Wels (*Silurus glanis*)	6	0.489	0.21	0.20	0.28	0.69
Pikepearch (*Stizostedion lucioperca*)	2	0.498	0.12	0.12	0.16	0.40
Pike (*Esox lucius*)	3	0.474	0.10	0.03	0.07	0.20
Perch (*Perca fluviatilis*)	2	0.490	0.71	0.17	0.91	1.79
Cormorant (*Phalacrocorax carbo*) - M	4	0.570	0.81	7.47	0.02	8.30
Cormorant (*Phalacrocorax carbo*) - L	4	0.570	1.36	18.25	0.00	19.62

The relative proportions of the HCH isomers varied widely across the species of the Danube Delta food web (Figure 1).
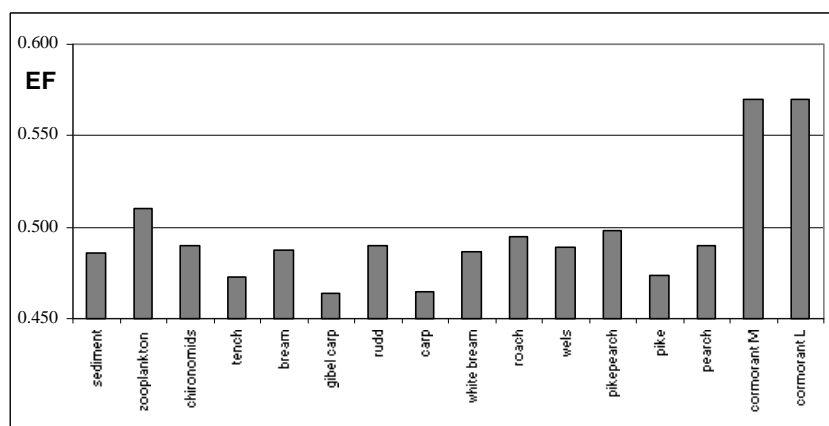
Figure 1. Relative proportions of HCH isomers in the Danube Delta food chain (invertebrates, fish and birds).

The profile of EFs (Figure 2) suggests a high species-dependency for the enantioselective accumulation of α-HCH, emphasising the importance of biological and ecological factors. Relatively low EF values were measured for invertebrates (zooplankton and chironomids) while a high selectivity for bird samples (muscle and liver) and a large species- and feeding mode-dependence for fish were observed[9]. A fish species-dependent enantioselective accumulation was recently also reported for various polycyclic musks. The relative proportions of the HCH isomers and EF for α-HCH in benthic invertebrates as well as in tench and bream (bentivorous fish) are similar to sediment (Figure 2), suggesting a minimal biotransformation of HCHs.
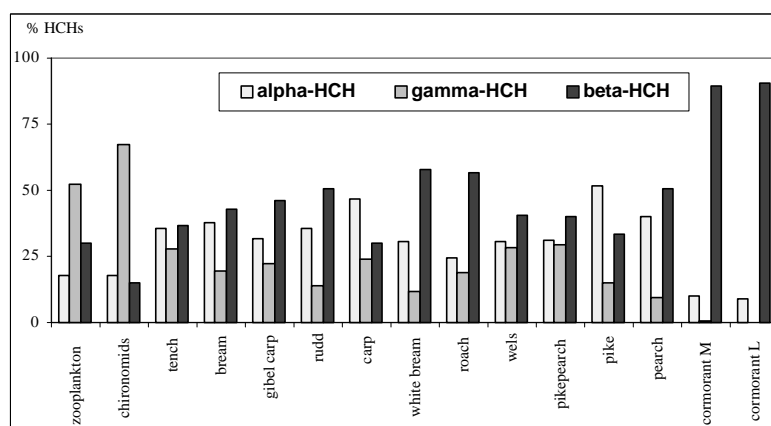

Figure 2. Profiles of EF for sediment and biota from the Danube Delta.

The toxicity and the fate of HCH isomers in humans, wildlife, plant, soil, water and atmosphere were recently reviewed[10]. α-HCH is the most stable isomer upon photolysis[11], which may explain its environmental persistence. Deo et al.[12] concluded that any HCH stereoisomer added to the environment is vulnerable to interconversion and degradation, processes which continue until an equilibrium is reached between different isomers. The reasons for differences in the enantiomeric enrichment of α-HCH in environmental and biological samples are still unclear.

The EF values were positively correlated with percents of β-HCH ($R^2=0.371$) and negatively correlated with percents of α-HCH ($R^2=0.5404$) for all studied species. Moisey et al.[13]

have suggested that the biotransformation and biodegradation of α-HCH results in a greater percentage of β-HCH, the most recalcitrant and bioaccumulable HCH isomer, indicating an increased metabolic capability. However, data from a large number of species (invertebrates, fish and birds) might result in a non-significant relationship.

The increase in the ratio of (+) α-HCH (the first eluting enantiomer) when going up in the food chain has also been observed for the polar bear food chain[14]. The ERs in higher trophic animals were influenced by species-specific metabolism and transport processes in the body as well as by biological factors, whereas the ERs were also changed by ecological factors such as feeding habits. Changes in ER due to differences in habitat and food sources were also found by Kallenborn et al.[15]. According to Vetter and Muraya[16] this is because in higher organisms pollutants are subjected to an increased specialization of enzyme systems. Deviations in ER, as a result of sexual maturity, aging and breeding activities were not significant[17].
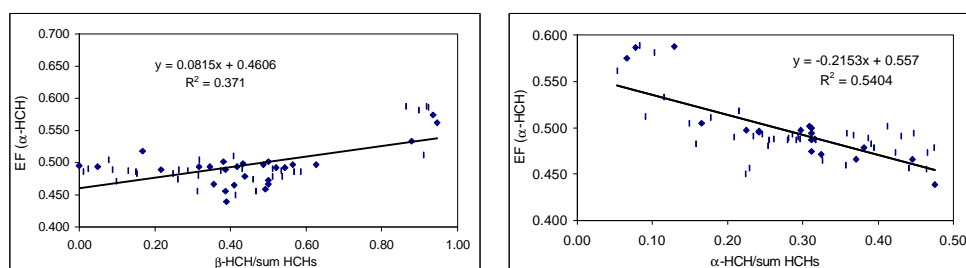

Figure 3. Relationship between EFs and percentage of β-HCH and α-HCH in an aquatic food chain from the Danube Delta, Romania.

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