CONTAMINATION OF FRESHWATER FISH FROM RIVERS SAVA AND DANUBE WITH POLYCHLORINATED BIPHENYLS

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ABSTRACT

During air strikes, in april 1999, Institute of meat hygiene and technology have begun examination of freshwater fish to establish the degree of contamination. The information about damaged industrial facilities and toxic waste that have been spilled were hard to find, and was unofficial and contradicts. Because of that, at the first time we collected samples from different locations, but after first results, we concentrated our attention on locations on river Danube downstream from Pančevo and on river Sava upstream from Belgrade, the locations indicated as environmental "hot spots". According to our experience, knowledge, equipment and analytical skills we have chosen to determine the concentrations of PCBs in freshwater fish species, since aquatic fauna might be used as indicator organisms for the evaluation of water pollution. Polychlorinated biphenyls as contaminant of interest, have been chosen because large quantities of PCBs reached the soil and waste and ground waters from damaged transformers and capacitors, where they serve as dielectric fluids. Also, PCBs are highly toxic and due to their liposolubility and persistence, these compounds accumulate through food chain. In 1999, from April to December, we had collected 23 samples of different fish species on river Danube, downstream from Pan~evo and 15 samples from locations on river Sava upstream from Belgrade. The concentrations of PCBs (mg/kg fat and mg/kg fresh weight) were expressed as the sum of individual congeners (IUPAC numbers 28, 52, 101, 138, 153, 180) and as Aroclor 1260 (peaks were identified as a fingerprint pattern by comparison with Aroclor standards). The concentrations of PCBs (mg/kg fat) are determined to evaluate the extent of contamination and concentrations of PCBs (mg/kg fresh weight) indicate daily intake and help us to estimate the risk for human health. Residues of PCBs in the fat extracted from fish sample were analysed according to the USDA Analytical Chemistry Guidebook. Gas chromatographs with electron capture detector (ECD) were used for analysis.

Key words: polychlorinated biphenyls, contamination, river, water, fish

INTRODUCTION

Polychlorinetd biphenyls (PCBs) are compounds for large seale inudstrial applications - for use in electrical industries in sealed systems as fluids for transformers and capacitors, as

additives to plastics or pesticide formulations, etc. The widespread production and use of PCBs in the 1960s and 1970s led, not only to environmental pollution close to sites of manufacture and usage, but, from that time started to be a great concern both for terrestrial and aqutaic environment[1]. Unproper use and disposal, as well as industrial accidents, due to persistence liposolubility and tendency of PCBs to bioaccumulate, resulted in their redistribution at sites remote from any production or use[2]. A long-term contamination of all components of the environment and of food chair was inevitable. Nowadays, they are of great concern because of their persistence in the environment, their tendency to bioaccumulate through the food chain and their toxic effects on wild life and humans[3].

In many cases it was inevitable that most of the primary products of PCBs, like oils from capacitors and transformers, find their way, as discarded materials, into the land and rivers, and consequently into the sea. In industrialised countries the main human intake of PCBs is via food consumption. Fish consumption, although relatively small in volume compared to other products, can contribute up to 50% of the intake of PCBs. Thus, the aquatic environment is the major route of PCBs entry to the food chain[4]. Fish and other sea food at all life stages, readily take up organochalogenated compounds, including PCBs, from water with rather high bioconcentration factors[5]. Accumulation of PCBs in fihs depends on their concentration in water, then on the life stage, specie and fat content of the fish[6]. From that point of view aquatic fauna might be used as indicator organisms for the evaluation of water pollution with PCBs. For that reason, from the time of air strikes in April 1999, we started to collect freshwater fish samples to establish the degree of contamination of fish species with PCBs, and indirectly to determine the degree of water pollution.

Our survey was undertaken to assess the contamination of fish species with PCBs from areas where the consequences of the air strikes on the environment was obvious. Polychlorinated biphenyls, as contaminants of interest, have been chosen because large quantities of PCBs reached the soil and waste and ground waters from damaged transformers and capacitors. Apart of that, we assumed that with time some quantities of the stocked PCBs might be discarded into running waters.

MATERIALS AND METHODS

In 1999., from April to December, 38 samples of different fish species (wels, catfish, pike, bream, bighead, crucian carp) have been collected. Sampling points were located on two places of special environmental concern: Danibe river, downstream from Pančevo and Sava river, upstream from Belgrade. After being cought, all fish specimens were kept frozen before analysis. Large fish were analysed as individuals. Small fish were analysed as composite samples of three to five fishes. Edible parts were chopped into 2-3 cm thick portions and homogenized. The appropriate portion of each sample was weighted for determination of fat content (in duplicate) and for analysis of PCB residues.

Fat content in edible parts of fish samples was detrmined by Soxhlet procedure. A weighted amount of sample was dried in oven at 103°C to a constant mass. The fat was then extracted in Soxhlet apparatus with light petroleum benzine. After evaporation of the solvent, the residual fat extract was dried to constant mass at 103°C.

Residues of polychlorinated biphenyls in the fat extracted from fish samples were analysed according to the USDA's Analytical Chemistry Laboratory Guidebook (1991). PCB's were extracted and separated by elution from fat in small glass columns filled with partially deactivated alumina. The eluate was evaporated to appropriate volume. A portion was injected into a gas chromatograph for detection and quantitation.

Two gas chromatographs (GC Varian Models 3400; Walnut Creck, CA, USA) were used for analysis of polychlorinated biphenyls in fat extracted from the fish samples. One was equipped with ^{63}Ni electron capture detector (ECD), and 1041 universal on column injector, a J&W DB-1 30 m long Megabore column (J&W Scientific), 0.53 mm i.d. and 1.5 μm film tickness. Operating conditions were as follows: injector 250°C; detector 300°C; column oven program: initial 80°C, hold 5 min, program at 5°C/min to 230°C, hold 15 min. The highly purified nitrogen carrier gas flow was 14 ml/min. The other Varian 3400 GC was equipped with a ^{63}Ni ECD and 1077 Split/Splitless capillary injector, and 30 m long Chrompack WCOT fused silica column CP SIL 8CB, 0.53 mm i.d. and 1.5 μm film tickness. Operating conditions: injector 250°C, injection mode splitless for 1 min; detector 300°C; column oven program: initial 80°C, hold 10 min, program at 5°C/min to 230°C, hold 20 min. Flow of highly purified nitrogen carrier gas was 8.5 ml/min.Data aquisition was performed by means of Varian integrator, Model 4 400.
The concentations of PCBs, presented as mg/kg fat and mg/kg fresh weight, were expressed as sum of individual congeners (IUPAC numbers 28, 52, 101, 138, 153 and 180) and as Aroclor 1260. PCB peaks in the examined samples were identified by comparison with individual congeners peaks and as a fingerprint pattern by comparison with different Aroclor standrads. The best match was obtained with Aroclor 1260. Oil samples taken from transformers destroyed during the air strike showed the fingerprint pattern of Aroclor 1260 too.
The accuracy of the obtained data was estimated from the recovery of PCBs (individual congeners and Aroclor 1260) from fortified samples. One blanck and two spiked samples, one with individual congeners and the other with Aroclor 1260 were included in each set of samples. The added quantities were in the range of concentrations expected for PCBs in the analysed samples. The results obtained for uncknown samples were corrected for recovery of individual congeners and Aroclor 1260 in spiked blank samples. Acceptable recoveries ranged from 80 to 100%.

RESULTS AND DISCUSSION

The content of PCBs (ranges and mean values) in edible tissues of 15 fish samples from Sava river, collected upstream from Belgrade, expressed as Aroclor 1260 and the sum of individual congeners is shown in table 1. The content of PCBs in edible tissues expressed as Aroclor 1260 and the sum of individual congeners ranged from 0.024 up to 0.524 mg/kg fat i.e. from 0.001 up to 0.043 mg/kg fresh weight. The mean values were 0.186 mg/kg fat i.e. 0.007 mg/kg fresh weight. The content of PCBs expressed as Aroclor 1260 were in the range of 0.380 - 5.150 mg/kg fat i.e. 0.008 - 0.177 mg/kg fresh weight. The mean values were 1.756 mg/kg fat i.e. 0.048 mg/kg fresh weight.
In table 2, the content of PCBs (range and mean values) in edible parts of 23 fish samples from Danube river, collected downstream from Pan~evo, expressed as Aroclor 1260 and the sum of individual congeners is shown. The content of PCBs in edible tissues expressed as Aroclor 1260 and the sum of individual congeners ranged from 0.018 up to 1.464 mg/kg fat i.e. from 0.001 up to 0.015 mg/kg fresh weight. The mean values were 0.308 mg/kg fat i.e. 0.006 mg/kg fresh weight. The content of PCBs expressed as Aroclor 1260 were in the range of 0.196 - 4.928 mg/kg fat i.e. 0.002 - 0.196 mg/kg fresh weight. The mean values were 1.809 mg/kg fat i.e. 0.056 mg/kg fresh weight.
When data from Table 1 and Table 2 are compared, the obtained higher value for sum of individual congeners in fish samples collected from Danube river indicate not only to a present but to a past exposition too. The content of PCBs expressed as Aroclor 1260 in fish

samples colleceted from both rivers, Danube and Sava, are very similar and indicate to a very present exposition to Aroclor 1260. This exposition might be a consequence of leakage from industrial sites of oil containing PCBs or a intentional discard of PCB oils in rivers. In the respect of the obtained results, the prevention of leakage or discard of PCBs, especially into rivers and other freshwater reservoires is very important for several reasons: rivers are a source of drinking water, freshwater fish form a relatively large proportion of the diet of the population and by flowing into sea, polluted rivers may contaminate the marine environment. Since the obtained results have indicated that fish, starting from May 1999, was exposed to a "present" contamination with PCBs we caried out our survey during the years 2000. and 2001. too. Since PCBs bioaccumulate during the years, we might expect that their concentration will increase by time. For that reason a permanent monitoring of PCBs concentrations in freshwater fish as bioindicators of water pollution with PCBs has to be performed in all places marked as "hot spots" of special environmental concern. A financial support for further investigations will be necessary.

Table 1. THE CONTENT OF PCBs IN FISH SAMPLES FROM SAVA RIVER, COLLECTED UPSTREAM FROM BELGRADE V-XII 1999.; 15 SAMPLES

		mg/kg fat	mg/kg fresh weight
Individual	range	0.024-0.524	0.001-0.043
kongeners	mean	0.186	0.007
Aroclor	range	0.380-5.150	0.008-0.177
1260	mean	1.756	0.048

Individual congeners - 28, 52, 101, 138, 153 and 180

Table 2. THE CONTENT OF PCBs IN FISH SAMPLES FROM DANUBE RIVER, COLLECTED DOWNSTREAM FROM PANČEVO V-XII 1999.; 23 SAMPLES

		mg/kg fat	mg/kg fresh weight
Individual	range	0.018-1.464	0.001-0.015
kongeners	mean	0.308	0.006
Aroclor	range	0.196-4.928	0.002-0.196
1260	mean	1.809	0.056

Individual congeners - 28, 52, 101, 138, 153 and 180

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