THE RESIDUES OF PCBs AND ORGANOCHLORINE HYDROCARBONS IN THE FROG LIVER FROM THE KRAGUJEVAC HOT SPOT

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Introduction

Anthropogenic environmental changes have caused serious deterioration of various natural ecosystems, as well as range reductions and population declines of many wildlife species, and many species have become extinct. Specific population declines are linked to habitat modification, or environmental pollutants may directly affect animal species. Persistent organic pollutants (POPs) represent the most hazardous environmental toxicants due to their ubiquity and long half-life, which provide long-term exposure and various chronic effects (1,2).

Polychlorinated biphenyls (PCBs) belong to the group of POPs. They were widely used in industry due to their chemical inactivity, thermal resistance, inflammability, low vapor pressure and high dielectric constant. Stockholm Convention (2001), which was signed by Serbia in 2002, totally banned the application of PCBs, which should be eliminated from any use until 2007. Although no longer manufactured and applied only with restrictions, PCB residues are still readily detectable in polluted sites and are found widely in animal tissues due to direct exposure or bioaccumulation.

Monitoring of PCB concentration in water, sediment and aquatic and terrestrial organisms, especially amphibians, is of a primary interest for determination of the level of pollution of aquatic and terrestrial ecosystem because of high bioconcentration factors of PCBs and their bioaccumulation in flora and fauna. Determination of PCBs in amphibians can also provide data on exposure of humans to these toxic contaminants, being the top of the food web.

Frogs may not be beautiful, but they can serve as very fine and very subtle pollution indicators, as it was shown in our previous investigations on pollution of Bor area (industrial town in Serbia, one of UNEPs hot spots in Serbia) (3).

The level of PCB contamination was significantly raised after the 1999 NATO attack to industrial facilities, when numerous transformers containing Pyralene oil were destroyed and PCBs leaked into soil and water flows. Kragujevac is the town in Central Serbia which was especially heavily targeted. The Zastava complex was hit twice during the Kosovo conflict. Heavy damage was inflicted on the power station, the car assembly line, the paint shop, the computer centre, and the truck plant. Some parts of the factory were completely destroyed.

During this conflict situation in Yugoslavia, bombing of oil refineries and electrical plants caused significant spills of transformers oils with high PCB concentration into water flows of the region.

Early stages of development in many amphibians occur in aquatic environment where toxins can be ingested via contaminated food or absorbed by dermal contact. Transport of PCBs through food web results in their bioaccumulation in frogs. Thus amphibians such as frogs play an important role in aquatic and terrestrial food webs by acting as both predator and pray. Characteristics of amphibian physiology and natural history (frogs are much less mobile animals) make them optimal and reliable bioindicators of the ecological state of an ecosystem (4,5). Recent studies suggest that amphibian populations are declining worldwide and that many environmental contaminants may be important factors in declines (7). Since frogs proved to be very sensitive and subtle bioindicators of both aquatic and terrestrial pollution, the levels of PCB contamination were determined in frog livers from the Kragujevac area.

Methods and Materials

Rana ridibunda.

Habitat & Biology: Strongly aquatic, inhabits lakes, pools or slowly flowing streams with much vegetation. Stays in close proximity of water bodies and prefers low plains or marshes. Sometimes seen in strong currents. A gregarious and diurnal species, but can forage also in the nighttime. Main diet is insects. A female lays 5,000-10,000 eggs as several masses either between aquatic plants or directly into open water. The species is widespread in Northern Africa, Middle and Southern Europe, Turkey, Western Asia. Genus *Rana* lives in both still and running waters. They inhabit an area in relatively large numbers; they live in different water and terrestrial systems, thus allowing direct comparison. All this makes them good bioindicators of terrestrial and aquatic ecosystems and can provide an earlier warning than other indicators.

Samples of frogs were collected from the banks of the lake Sumarice, which was formed in Memorial Park by accumulation of natural well. The lake covers area of 14.000m^2 with volume of 950.000m^3. This artificial lake was inhabited with different kinds of fishes. Some kinds of ducks, herons and other birds could be found in the lake surroundings. Today, it is a well-known tourist resort.

Samples of *Rana ridibunda* species (12 specimens) were collected in September 2002. PCBs were extracted from frog livers with the mixture of chloroform:methanol:water (2:2:1). Organic extract was purified in the separatory funnel with concentrated sulfuric acid. Content of PCBs was determined by gas chromatography (GC/ECD).

Parameters of gas chromatographic determination:

Instrument: HP 6890 gas chromatograph
Column: capillary, HP-5, 60m x 0.32mm x 0.25µm,
Temperature regime: Initial temp: 130^{o}C, 2 min, T 2: 150^{o}C, 5 min, Final T: 295^{o}C
Detector: ECD, 310^{o}C
Standard: Dr. Ehrenstorfer standard mixture of organochlorine pesticides and polychlorinated biphenyls 18001300 Pesticide-Mix 13.

Results and Discussion

R. ridibunda is a non-hybrid sensitive species reacting strongly to the environmental pollution, which results in various abnormalities and malformations of limbs, described by Puky and Fodor (6) for frogs *R. esculenta* and *B. bombina* in the Hungarian part of the Danube, Tisza and Ipoly, especially in the flooded areas. Since there was a great flood in the Kragujevac region a few months after the war accidents, it can be expected that all pollutants with density lower than

water are accumulated in the soil of the flooded areas, which would probably result in the higher levels of PCBs in livers of frogs from this locality.
Although the Lake of Sumarice is relatively distant from area targeted during war accidents, organochlorine contaminants, especially PCBs, were detected in frog samples. Almost all EPA's indicator PCB congeners, except PCB 52, were detected in all frog liver samples (Table 1.) There is no literature data on the maximal allowed concentrations of PCBs in amphibians; however, frog meat is used in human diet, and the residual limits should be established for frogs.

Table 1. Content of non-dioxin like PCB congeners detected in frog livers

PCB congener	Range (ng/g liver)	Average content (ng/g liver)
PCB 28	0.9840 - 1.1702	1.0771
PCB 52	ND	ND
PCB 101	ND – 0.3196	0.1598
PCB 153	0.5437 – 2.5207	1.5322
PCB 138	0.7864 – 1.9829	1.3846
PCB 180	0.5195 – 1.2661	0.8928
TOTAL		**5.0465**

Figure 1: Content of non-dioxin like PCB congeners detected in frog livers

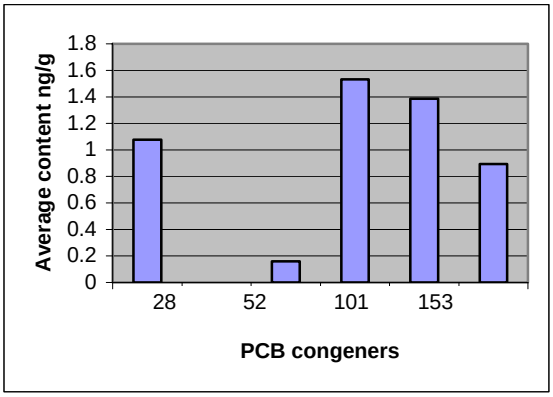
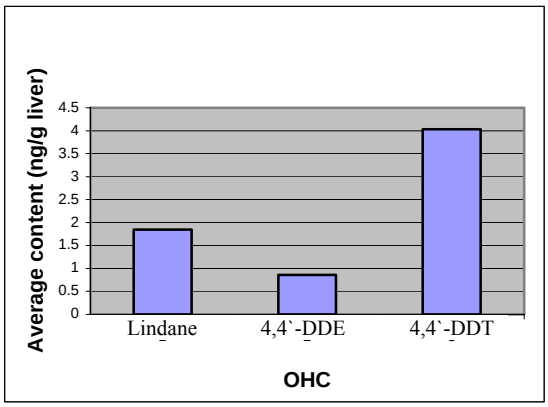


Table 2. Content of organochlorine compounds detected in frog livers

Organochlorine contaminant	Range (ng/g liver)	Average content (ng/g liver)
Lindane	1.7891-1.9154	1.8429
4,4`-DDE	0.3579-1.7134	0.8561
4,4`-DDT	2.1425-5.4280	4.0310

Figure 2. Content of organochlorine compounds detected in frog livers



Detected organochlorine insecticides, especially PCBs in the Kragujevac locality indicate both historical and more recent pollution, where the dominant way of PCBs input is probably atmospheric transport.

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