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COMPARATIVE RESULTS OF THE RESIDUAL LEVELS OF PCBs OBTAINED BY GC/ECD AND BIOASSAY IN SOIL SAMPLES FROM KRAGUJEVAC HOT SPOT AFTER THE WARFARE IN FORMER YUGOSLAVIA

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Introduction

In the last decades, persistent organic pollutants (POPs) have become a major issue of research in order to investigate their ubiquitous environmental occurrence, biochemical and toxic effects, human exposure and health risk assessments. Some of these have been produced intentionally (PCBs) in a wide variety of commercial applications because of their excellent technological or pesticide properties. Other persistent and very toxic pollutants such as PCBs, dioxins and furans have been formed as undesirable by-products.

During Kosovo intervention in 1999, almost daily attacks on major industrial sources caused numerous industrial accidents (Kragujevac, Pancevo, Novi Sad, Bor etc.)[1]. The main environmental effects of the bombardments in Kragujevac, ZASTAVA car factory (Paint Shop, Power Plant, Waste Storage) were damaged transformers which contained Pyralene oil. As a consequence of explosions, fires and PCB combustions, very high levels of PCDD/F were also found in soil samples from that area (100,000 ng I-TEQ/kg). Very high levels of PCBs and PCDD/Fs were also found in samples taken around the transformers of the power plant (70-74 g/kg of PCBs and 10,200 ng I-TEQ/kg of PCDD/Fs).

In 2000, UNEP/UNOPS Clean-up Project YUG 00-R71 was performed for clean-up action of serious environmental damage of hotspots in Former Yugoslavia.

The aim of this work was to identify the potentially polluted zone in the vicinity of the destroyed transformers in Kragujevac non-urban area.

Methods and Materials

In December 2002, the sampling of soil was conducted at the localities which were supposed to be still contaminated with PCB[2,3]. All the sampling spots are situated on the base of destroyed transformer station. Sampling zone (12x11m) was divided in quadrants and soil samples were

taken from the various depths (0.2 – 1.0 m) (Fig. 1.). Sampling locations were denoted as: A-1(0.4 m), B-2(0.6 m), B-1(0.2 m), B-4(0.4 m), B-3(0.2 m), B-5(1.0 m) and C-1 (0.2 m).

Residual quantities of PCBs in soil were identified qualitatively by *in vivo* bioassey and then determined by GC/ECD analysis.

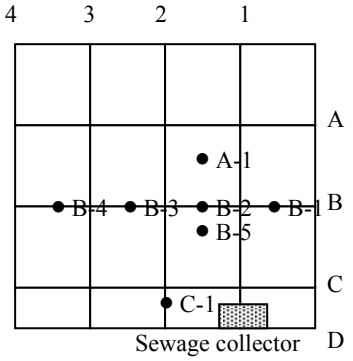

Figure 1. Sampling zone

GC/ECD. Seven sediment samples (50 g each) were extracted by 100 ml of acetone:hexane mixture (1:1) on the magnetic stirrer for 24 h. After evaporation till dryness, lipophilic residues were dissolved in 5 ml hexane and purified with concentrated sulfuric acid until discoloration. The samples were rinsed with water and evaporated[4]. The residue was dissolved in 1 ml hexane and concentrations of PCBs were determined by gas chromatography.

Parameters of gas chromatographic determination:
Instrument configuration: HP 6890 gas chromatograph
Working temperature: initial temp. 130 °C, maximum temp. 300 °C
Column: Capillary column HP 19091J-416 HP-5 5% Phenyl- Methyl-Siloxane
Detector: Electron capture detecor (Varian 3400)
Standard: Riedel-de Haën standard mixtures of 7 congeners (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180, PCB 209)

Biological assay. Four sediment samples (50 g each) were extracted by 100 ml of acetone:hexane mixture (1:1) on the magnetic stirrer for 24 h. Crude extracts were evaporated and dissolved in olive oil to achieve the desired concentration. Immature female rats (20 days old) were injected intraperitonealy (ip) with soil extract, or with olive oil alone successively for 3 days. 24 hours after the last injection, animals were sacrificed and hepatic microsomal fraction were isolated by differential centrifugation described in Li et al[5]. Hepatic ethoxyresorufin O-deethylation assay (EROD) and pentoxyresorufin O-deethylation assay (PROD) activities were determined spectrofluorimetrically based on the method of Burke et al[6] with some modification. Reaction mixture contained 0.05 M Tris-HCl pH 7.4, 2.5 :M ethoxyresorufin, 9.5 :M penthoxyresorufin, microsomal suspension (100 :g protein for EROD and 300 :g for PROD) and NADPH-regenerative system. The reaction was carried out at 37^0C and stopped by addition of 2 ml

methanol after 5 min for EROD and 25 min for PROD activity. The amount of formed resorufin was detected by measuring sample fluorescence with excitation at 550 nm and emission at 585 nm relative to known amount of resorufin (range: 2.44 nM to 78 nM). This *in vivo* bioassay is based on female integrated endocrine disruption assay (FRIEDA) that can be used as a rapid screening tool for investigation of the presence of chemicals with potential dioxin-like and non-dioxin-like effects in different environmental matrices[5].

Results and Discussion

The results of both analytical methods and bioassay conducted in this study show that in spite of clean-up and decontamination of Kragujevac car factory, residues of PCBs are still found in soil samples from that area.

Results of GC/ECD analysis of soil samples are given in Table 1.

Table 1. Content of PCBs in soil samples

PCB congener	Concentration of PCBs in different sampling sites (ng/g)						
	A-1 (0.4 m)	B-1 (0.2 m)	B-5 (1.0 m)	C-1 (0.2 m)	B-2 (0.6 m)	B-3 (0.2 m)	B-4 (0.4 m)
PCB 28	-	-	-	-	-	-	-
PCB 52	2.27e+2	1.22e+2	7.08e+1	6.23e+1	4.61e+1	2.31e+1	1.10e0
PCB 101	1.01e+3	7.84e+2	3.15e+2	2.69e+2	1.92e+2	1.00e+2	8.46e0
PCB 153	7.89e+2	6.47e+2	2.28e+2	1.86e+2	1.26e+2	7.62e+1	9.78e0
PCB 138	1.44e+3	1.20e+3	4.85e+2	3.69e+2	2.51e+2	1.47e+2	1.61e+1
PCB 180	2.06e+2	1.70e+2	7.13e+1	6.03e+1	3.78e+1	2.94e+1	5.38e0
TOTAL	3.67e+3	2.92e+3	1.17e+3	9.46e+2	6.53e+2	3.77e+2	4.08e+1

In all soil samples 5 PCB congeners were detected. Congener PCB 28 was identified in samples A-1 and B-1, but it could not be quantitatively determined.

According to the results of GC analysis, the center of contamination is the spot A-1, while the most polluted area is determined by spots A-1, B-1 and B-5, regardless the different sampling depths. Presence of PCBs in spot B-5 (1.0 m) indicates their vertical movement into soil, which, according to hydrogeological model (Matic, unpublished) could predict even deeper migration of PCBs. In spite of limited data, it can be suggested that the vertical migration of PCBs in soil is more pronounced than horizontal.

The results of *in vivo* bioassay show that all four samples significantly induced EROD activities in immature female rats in a dose-dependent manner. The samples A-1 and B-1 were the most potent, which is in accordance with their high PCB contamination, as determined by GC/ECD. On the other hand, PROD activities were not significantly increased, except with sample A-1 (Fig. 2) which suggests that concentration of ortho-substituted PCBs in soil is below detection limit of bioassay.

According to GC/ECD analysis soil sample A-1 contains PCBs in concentration of 3.67 mg /kg soil, what means that actual dose of PCBs injected in rats was 0.62 mg/kg body weight. However, such dose is lower that NOEL for Pyralene: 10 mg/kg body weight in *in vivo* bioassay used in this study (unpublished observation). On the other hand, with extracts from 6.8 g of sample A-1,

EROD activity was 2.5 times higher comparing to control. It is similar to the effect of Pyralene in the dose of 15-30 mg/kg body weight which doubled EROD activity in comparison to control[7]. Also, results of Li et al.[5] demonstrated that extract of soil samples in the dose of 10 mg/kg body weights are able to induce significant increase in EROD activity in the same *in vivo* bioassay as used in this study.

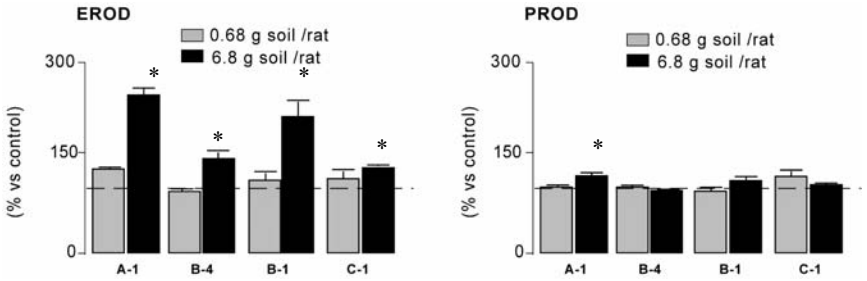

Fig. 2. Effects of different soil extract on EROD and PROD activities in liver microsomes of immature female rat. Columns represent means ± SE from 5 animals per group. Legend in figure represent total amount of soil extract received for 3 days. Basal EROD and PROD levels were 35.98±3.41 and 2.165± 0.158 pmol/min/mg prot respectively. Significance: *p<0.05 using ANOVA followed by Duncan's test

Taking into account above mentioned observations it could be suggested that relatively high EROD induction capacity of soil samples could be result of the presence of dioxin-like compounds, or some other arylhydrocarbon receptor agonists in the investigated samples, which is in accordance with the results of Balkan Task Force study[1] concerning dioxin presence in the area immediately after the accident.

In conclusion, application of combined bioassay and GC techniques is the optimal procedure for identification and detection of PCBs and other dioxin like compounds in abiotic and biotic matrix.

Acknowledgements
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