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# Distribution of Organochlorine Compounds in Pine Needles Collected in Zagreb

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**Abstract** The distribution of polychlorinated biphenyls and organochlorine pesticides was investigated in one- and two-year-old pine needles collected at eight locations in Zagreb in 2006. Concentration medians for most analyzed compounds in two-year-old needles were higher or similar to the concentration medians of the same compounds in one-year-old needles, with the exception of PCB-28, PCB-52, PCB-153, and PCB-138. The pollution profiles seems to have remained the same over the 2 years. These results were compared to those obtained in samples collected at the same locations in 1998.

**Keywords** PCBs · Organochlorine pesticides · Conifer · Environmental pollution

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) belong to persistent, organic pollutants. Although their use was banned or restricted in the 1970s due to harmful effects on human and animal health, because of their persistency and bioaccumulation they are still present in all parts of the environment.

Pine needles are considered to be among the best passive indicators of environmental pollution with organochlorine compounds because of their global distribution and waxy surface which accumulates lipophilic compounds from the surrounding air (Jensen et al. 1992; Wenzel et al. 1997).

This study investigated the distribution of twenty PCB congeners and several OCPs in one- and two-year-old pine needles collected at eight locations in Zagreb, Croatia. These levels were compared to those measured in samples collected at the same sites in 1998.

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## Materials and Methods

*Pinus strobus* and *Pinus nigra* needle samples were collected in March 2006 at eight sites in Croatian capital Zagreb with the population of about one million. Branches were collected at approximately 1.5 m above the ground level and stored in plastic bags. One- and two-year-old needles were manually separated and dried at room temperature to constant weight.

Twenty PCB congeners were analyzed: PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180, PCB-77, PCB-105, PCB-114, PCB-118, PCB-123, PCB-126, PCB-156, PCB-157, PCB-167, PCB-169, PCB-170, PCB-189, PCB-60, and PCB-74 (numbered according to IUPAC), and the following organochlorine pesticides: hexachlorobenzene (HCB), α-HCH, β-HCH, γ-HCH (α-, β-, γ-hexachlorocyclohexanes), 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene (DDE), 1,1-dichloro-2,2-di(4-chlorophenyl)ethane (DDD) and 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT).

Dried ground needles (5 g) were mixed with dichloromethane (20 mL) in a Teflon PFA (perfluoroalkoxy) extraction vessel (GreenChem Plus) and extracted (MAE) using the Microwave Accelerated Reaction System for Extraction MarsX (CEM, USA) at 1200 W and 40°C for 15 min. The extract was filtered through a dichloromethane prewashed filter paper (Whatman no. 1) and evaporated to dryness under a gentle stream of nitrogen.

Extract cleanup is a multi-step procedure because needle wax has a complex composition with many compounds that can interfere with determination of PCBs and OCPs. The

extract was cleaned by alkaline and acidic hydrolysis and by adsorption chromatography on a multilayer silica column. The whole procedure has been described by Herceg Romanić and Krauthacker (2004, 2006). After adsorption chromatography, the eluate was reduced to dryness and redissolved in *n*-hexane for gas chromatographic analysis.

High resolution gas chromatography with electron capture detector(s) (HRGC/ECD) was done using at "ATI UNICAM" 610 SERIES chromatograph with two $^{63}$Ni detectors. The compounds were analyzed simultaneously on two capillary columns ("Supelco", SAD): (1) 60 m × 0.25 mm, SPB-5 film thickness 0.25 µm, temperature programme 100°C, then 4°C min$^{-1}$ to 240°C, 50 min isothermally; and (2) 30 m × 0.25 mm, SPB-1701 film thickness 0.25 µm, temperature programme 110°C, then 4°C min$^{-1}$ to 240°C, 50 min isothermally. Carrier gas was nitrogen. The injector and detector temperature were 250°C and 270°C respectively, and the volume of injected sample was 5 µL. Qualitative and quantitative analyses were done by comparison with external standards. Each sample was analyzed on both columns and only compounds identified on both columns were evaluated. The detection limit was 0.4 ng mL$^{-1}$ for each compound.

## Results and Discussion

The distribution of organochlorine compounds was investigated in one- and two-year-old pine needles collected in March 2006 at eight locations in Zagreb, seven of which were residental [north (Ksaver), north east (Markuševačka Trnava), west (Savski Gaj, Savska Opatovina), south (Odra), east (Borongaj and Kraljevački Novaki)], and one industrial, near the waste dump (Jakuševac). Table 1 shows the incidence of compounds in the samples.

Ockenden et al. (1998) have concluded that for the proper comparison of the results of analyses of samples from different locations it is necessary to collect samples of the same (or possibly taxonomically very similar) species and age. *Pinus strobus*, collected only at location Odra, and *Pinus nigra*, collected at other locations in our study, are of the same species.

In all samples the following compounds were found: HCB, α-HCH, β-HCH, γ-HCH, DDE, DDT, indicator congeners of PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180), and PCB-60. PCB-77, PCB-123, PCB-126, PCB-156, and PCB-169 were not found in any of the samples. In one-year-old needles indicator PCBs ranged between 0.31 ng g$^{-1}$ and 4.45 ng g$^{-1}$ dry weight, and the rest of PCB congeners in positive samples between 0.17 ng g$^{-1}$ and 1.44 ng g$^{-1}$ dry weight. Indicator PCBs in two-year-old needle samples ranged between 0.27 ng g$^{-1}$ and 7.20 ng g$^{-1}$ dry weight, and the rest of PCBs in positive samples between 0.17 ng g$^{-1}$ and 2.10 ng g$^{-1}$ dry weight. OCPs in one-year-old needle samples ranged between 0.08 ng g$^{-1}$ and 5.51 ng g$^{-1}$ dry weight, and in two-year-old needle samples between 0.26 ng g$^{-1}$ and 7.15 ng g$^{-1}$ dry weight.

**Table 1** Concentration ranges of organochlorine compounds in one- and two-year-old dry pine needles collected in Zagreb

| Compound | One-year-old needles (N = 8) | | Two-year-old needles (N = 8) | |
|---|---|---|---|---|
| | w/ng g$^{-1}$ | n | w/ng g$^{-1}$ | n |
| HCB | 0.33–0.80 | 8 | 0.36–1.07 | 8 |
| α-HCH | 0.08–0.91 | 8 | 0.29–1.18 | 8 |
| β-HCH | 2.21–5.51 | 8 | 2.16–7.15 | 8 |
| γ-HCH | 0.1–1.03 | 8 | 0.64–1.73 | 8 |
| DDE | 0.24–0.49 | 8 | 0.26–0.64 | 8 |
| DDD | 0–0.36 | 1 | 0–0.44 | 2 |
| DDT | 0.53–0.79 | 8 | 0.43–0.90 | 8 |
| PCB-28 | 1.94–3.48 | 8 | 1.75–4.19 | 8 |
| PCB-52 | 1.73–4.45 | 8 | 1.47–7.20 | 8 |
| PCB-101 | 1.17–2.19 | 8 | 1.04–2.19 | 8 |
| PCB-138 | 0.94–1.34 | 8 | 0.65–1.20 | 8 |
| PCB-153 | 0.77–1.12 | 8 | 0.48–0.99 | 8 |
| PCB-180 | 0.31–0.44 | 8 | 0.27–1.05 | 8 |
| PCB-60 | 0.45–1.44 | 8 | 0.60–2.10 | 8 |
| PCB-74 | 0–0.53 | 5 | 0–0.58 | 8 |
| PCB-77 | 0 | 0 | 0 | 0 |
| PCB-105 | 0–0.38 | 2 | 0.26–1.10 | 8 |
| PCB-114 | 0–0.44 | 4 | 0.23–0.51 | 8 |
| PCB-118 | 0.37–0.67 | 8 | 0–0.77 | 6 |
| PCB-123 | 0 | 0 | 0 | 0 |
| PCB-126 | 0 | 0 | 0 | 0 |
| PCB-156 | 0 | 0 | 0 | 0 |
| PCB-157 | 0–0.31 | 3 | 0.17–1.31 | 8 |
| PCB-167 | 0 | 0 | 0–0.33 | 7 |
| PCB-169 | 0 | 0 | 0 | 0 |
| PCB-170 | 0–0.35 | 6 | 0–0.36 | 7 |
| PCB-189 | 0–0.17 | 1 | 0–0.25 | 6 |

N – number of samples; n – number of positive samples; 0 – below detection limit

Figure 1 shows that the concentration medians of most analyzed compounds in two-year-old needle samples are higher than or similar to those in one-year-old needle samples. This was expected considering that two-year-old pine needles accumulated more pollutants over that time.

However, the concentration medians of indicator congeners PCB-28, PCB-52, PCB-153, and PCB-138 are slightly higher in one-year-old needle samples than in two-year-old needle samples. Concentration medians of OCPs in one-year-old needle samples appear in the following order: β-HCH > γ-HCH > DDT > HCB > α-HCH > DDE, and in

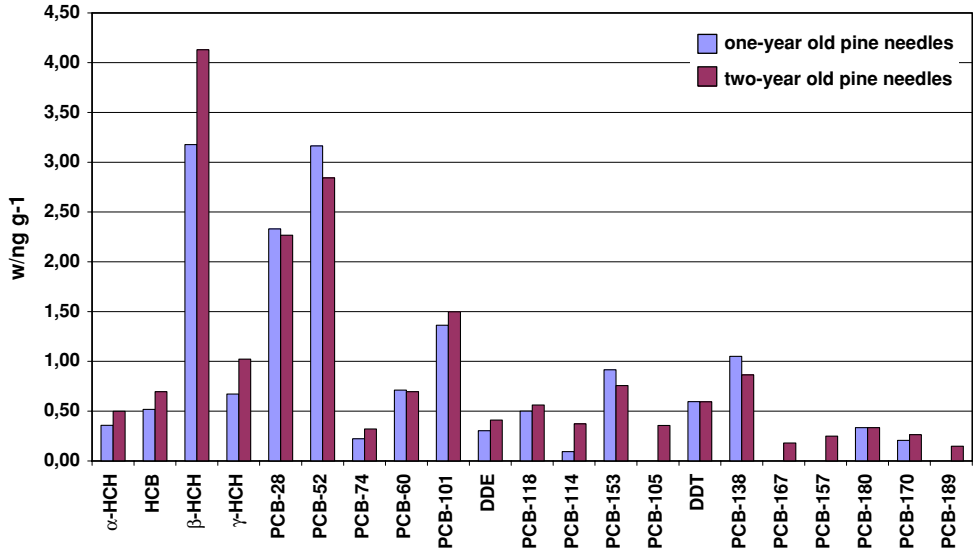


**Fig. 1** Median concentrations of PCBs and OCPs in one- and two-year-old dry pine needle samples collected in Zagreb

two-year-old needle samples as follows: $\beta$-HCH > $\gamma$-HCH > HCB > DDT > $\alpha$-HCH > DDE. The concentration medians of PCBs in one-year-old needle samples appear in the following order: PCB-52 > PCB-28 > PCB-101 > PCB-138 > PCB-153 > PCB-60 > PCB-118 > PCB-180 > PCB-74 > PCB-170 > PCB-114 (concentration medians for PCB-105, PCB-167, PCB-157, and PCB-189 were 0) and in two-year-old needle samples: PCB-52 > PCB-28 > PCB-101 > PCB-138 > PCB-153 > PCB-60 > PCB-118 > PCB-114 > PCB-105 > PCB-180 > PCB-74 > PCB-170 > PCB-157 > PCB-167 > PCB-189. These similarities in concentration medians of analyzed compounds between one- and two-year-old needles indicate the same pollution profile in 2 years.

The results obtained in this study were compared to those reported by Herceg Romanić and Krauthacker (2004) for 1998 (see Table 2). To be able to compare results, levels in the needles collected in 2006 are presented as means of levels found in one- and two-year-old needles because in the earlier study by Herceg Romanić and Krauthacker (2004) one- and two-year-old needles were mixed and analyzed together. Over the eight years between 1998 and 2006, the levels of organochlorine compounds in pine needles did not significantly decrease. On almost every location $\beta$-HCH, DDT, PCB-28, and PCB-52 levels increased, while $\gamma$-HCH, DDE, and DDD decreased. PCB-153 and PCB-180 levels did not change. The 1998 OCP concentration medians appeared in the following order: $\gamma$-HCH > $\beta$-HCH >

**Table 2** Concentrations (w/ng $g^{-1}$ dry weight) of organochlorine compounds in pine needle samples collected at same locations in Zagreb in 1998 and 2006

| Location/year | HCB | α-HCH | β-HCH | γ-HCH | DDE | DDD | DDT | PCB-28 | PCB-52 | PCB-101 | PCB-138 | PCB-153 | PCB-180 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B./1998 | 1.38 | 0.44 | 0.57 | 1.59 | 0.35 | 0.22 | 0.72 | 1.74 | 5.09 | 1.96 | 1.76 | 0.74 | 0.56 |
| 2006 | 0.94 | 0.94 | 5.30 | 1.29 | 0.56 | 0.00 | 0.81 | 3.70 | 5.82 | 1.91 | 1.27 | 1.06 | 0.69 |
| O./1998 | 0.70 | 0.33 | 2.10 | 1.13 | 0.66 | 0.22 | 0.14 | 2.52 | 0.34 | 1.79 | 0.56 | 0.50 | 0.16 |
| 2006 | 0.61 | 0.42 | 2.84 | 0.37 | 0.40 | 0.00 | 0.72 | 2.66 | 2.39 | 1.30 | 1.00 | 0.87 | 0.33 |
| K. N./1998 | 1.00 | 1.15 | 2.84 | 5.25 | 0.84 | 0.29 | 0.33 | 1.33 | 0.78 | 1.45 | 0.92 | 0.54 | 0.12 |
| 2006 | 0.69 | 0.59 | 3.88 | 1.37 | 0.32 | 0.40 | 0.59 | 3.15 | 2.09 | 1.40 | 0.95 | 0.76 | 0.32 |
| Od./1998 | 0.69 | 0.39 | 2.03 | 2.17 | 1.45 | 0.21 | 0.41 | 1.64 | 0.56 | 2.32 | 1.65 | 0.79 | 0.24 |
| 2006 | 0.85 | 0.22 | 3.45 | 0.81 | 0.26 | 0.12 | 0.52 | 2.27 | 2.61 | 1.49 | 0.95 | 0.70 | 0.32 |
| M. T./1998 | 0.64 | 0.63 | 3.01 | 3.80 | 0.72 | 0.32 | 0.23 | 3.71 | 0.42 | 2.96 | 1.74 | 0.41 | 0.35 |
| 2006 | 0.54 | 0.34 | 3.94 | 0.83 | 0.32 | 0.00 | 0.54 | 1.88 | 3.12 | 1.19 | 0.84 | 0.75 | 0.37 |
| K./1998 | 0.83 | 0.97 | 1.14 | 3.63 | 0.64 | 0.44 | 2.62 | 1.58 | 2.80 | 2.72 | 0.69 | 0.74 | 0.48 |
| 2006 | 0.47 | 0.30 | 2.18 | 0.74 | 0.38 | 0.00 | 0.80 | 2.02 | 2.98 | 1.95 | 0.93 | 0.78 | 0.34 |
| J./1998 | 1.55 | 1.27 | 0.96 | 4.91 | 0.72 | 0.50 | 2.65 | 0.68 | 6.63 | 1.63 | 1.23 | 1.30 | 0.54 |
| 2006 | 0.38 | 0.29 | 3.04 | 0.66 | 0.26 | 0.00 | 0.50 | 2.23 | 2.99 | 1.42 | 0.91 | 0.88 | 0.34 |

Locations: B. = Borongaj; O. = Opatovina; K. N. = Kraljevački Novaki; Od. = Odra; M. T. = Markuševačka Trnava; K. = Ksaver; J. = Jakuševec

HCB > DDE > α-HCH > DDT > DDD, and in 2006 samples as follows: β-HCH > γ-HCH > HCB > DDT > α-HCH > DDE > DDD. The 1998 concentration medians of indicator PCBs appeared in the following order: PCB-101 > PCB-28 > PCB-138 > PCB-52 > PCB-153 > PCB-180, and in needles collected in 2006: PCB-52 > PCB-28 > PCB-101 > PCB-138 > PCB-153 > PCB-180. This comparison shows some changes in the distribution of OCPs and PCBs. For instance, β-HCH, DDT, and PCB-52 concentration medians increased over the eight years. As mentioned earlier, a comparison of organochlorine compound levels in 1998 and 2006 needles shows that β-HCH, DDT, PCB-28, and PCB-52 levels increased and PCB-153 and PCB-180 levels remained unchanged. The same was noted in one- and two-year-old needles (Fig. 1), where concentration medians of PCB-28, PCB-52, PCB-153, and PCB-138 were slightly higher in one- than in two-year-old needle samples.

α-HCH/γ-HCH and DDE/DDT ratios are often used to indicate fresh input of γ-HCH or DDT in the environment. α-HCH/γ-HCH ratios are lower than 1 in all samples except in the sample from Opatovina, which indicates a fresh input of γ-HCH. In comparison with 1998, ratios in the samples from 2006 increased, which is in agreement with the γ-HCH decrease over the eight years (Table 2).

In all samples collected in 2006 DDE/DDT ratios were lower than 1, whereas the 1998 ratios were higher than 1 in four samples. Consequently, median of DDE/DDT ratios (ranges of ratios DDE/DDT in individual samples: 0.24–4.89 in 1998 and 0.47–0.70 in 2006) were lower in 2006 than in 1998 (0.55 and 2.55, respectively). All that data suggest that there is a fresh input of DDT in our environment, and the same conclusion was given earlier by Herceg Romanić and Krauthacker (2004). DDE/DDT ratios may have decreased due to long-range transport, perhaps from Africa or other areas where these compounds may still be in use against malaria or from Italy where DDT may have accidentally leaked (Di Guardo et al. 2003).

There are not many published data about the levels of organochlorine compounds in pine needles collected at urban sites, especially over the last few years. Hellström et al. (2004) analyzed organochlorine pesticides in different year classes of pine needles from Central and Northern Europe collected in 1989 and 1990. The levels of α-HCH and HCB were relatively uniform throughout Europe, while the levels of γ-HCH and the DDT group showed higher levels in the southern parts of the investigated area. Wenzel et al. (1997) analyzed five PCB congeners (PCB-28, PCB-52, PCB-101, PCB-138, and PCB-153) and organochlorine pesticides in two-year-old pine needles collected in two urban parks in Mendoza (Argentina) in April/May 1994 and at 20 sites in the Leipzig-Halle region (Germany) in January–March 1993. The levels of organochlorine pesticides in urban Mendoza parks ranged between 0.3 ng $g^{-1}$ dry weight for DDD and 1041 ng $g^{-1}$ dry weight for β-HCH and in region Leipzig–Halle between 0.5 ng $g^{-1}$ dry weight for DDD and 17 ng $g^{-1}$ dry weight for α-HCH. Levels of analyzed PCBs ranged between below the detection limit (PCB-138) and 5.9 ng $g^{-1}$ dry weight (PCB-138) in urban Mendoza parks and between 0.3 ng $g^{-1}$ dry weight (PCB-52 and PCB-153) and 1.3 ng $g^{-1}$ dry weight (PCB-138) in the Leipzig–Halle region. The levels of organochlorine compounds in pine needles collected in Zagreb, Croatia's capital with population of about one million, are within the lower part of the concentration range of concentrations measured in needles collected in different parts of Europe.

Our results show that the levels of organochlorine compounds are not decreasing at the expected rate due to restricted use. On the contrary, the levels of some organochlorine compounds have increased over the eight years, which can be explained by a fresh input and/or long-range transport of these persistent compounds.

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