NON-DESTRUCTIVE BIOMONITORING OF POPs AND PBDEs IN MAGPIES (*PICA PICA*): COMPARISON OF FEATHERS, SERUM AND PREEN GLAND

Jaspers V L B[1], Deleu P[1], Covaci A[2,3], Neels H[2], Eens M[1]

1- Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
2- Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
3- Ecophysiology Biochemistry & Toxicology, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp

Abstract

In this study, we examined the levels of POPs and PBDEs in tail feathers, serum and the preen gland of magpies (*Pica pica*). High and significant correlations (Spearman rank correlation coefficient (r_s) up to 0.9 and $p < 0.001$) were established between concentrations of selected organic pollutants in tail feathers and concentrations in the preen gland of magpies. Correlations of feathers and serum were only significant for the higher chlorinated PCB congeners. The results of this study suggest that preening feathers with oil from the uropygial gland could have an important impact on concentrations of organic compounds measured in the feathers.

Introduction

Recently feathers have been put forward as a potential non-destructive biomonitoring tool for organic pollutants[1,2]. One tail feather of a buzzard was found to be sufficient to quantify concentrations of most PCBs, PBDEs and DDTs[1]. Moreover, significant positive correlations were found between the concentrations in the feathers and levels measured in internal tissues (muscle or liver) [1,2]. For heavy metals, external contamination has been shown to have an important influence on the concentrations measured in the feathers, depending on the metal under consideration[3,4]. Two routes of external contamination have been distinguished for heavy metals[5,6]: external contamination by wet or dry deposition of chemicals via the air, or external contamination through preening feathers with oil from the uropygial gland (preen gland). Recently, a first study was carried out to investigate the extent of external contamination with organic pollutants via the air[7]. The authors concluded that external contamination with organic pollutants via the air is probably of small importance[7]. However, as lipophilic chemicals have the ability to accumulate at high concentrations into oily secretion, preening feathers could present an important route of external contamination onto feathers.

In this study, we examined the levels of POPs (PCBs and OCPs) and PBDEs in tail feathers, serum and the preen gland of magpies (*Pica pica*). All these methods can be categorized as non-destructive sampling methods. We carried out correlations between levels in feathers and serum on the one hand, and between levels in feathers and preen gland on the other hand. Thus, we could investigate internal (via the bloodstream) versus external contamination (via preening) with organic pollutants in feathers. Furthermore, these three sampling methods were compared as regards their usefulness and predictability in monitoring of organic pollutants.

Materials and Methods

Sample collection - In the autumn of 2006, 12 magpies were captured at the Campus Drie Eiken of the University of Antwerp using a Larsen trap (Group A). From these birds, a blood sample (0.5 – 1 ml) was taken and the third tail feather was pulled on both sides. The blood was centrifuged (10 min at 8 500 rpm), and the serum (ca. 0.5 ml) was stored at -20°C for later analysis. After sampling, the birds were released. In addition, sixteen cadavers of magpies were obtained via Wildlife Rescue Centres and the local hunting association (Hubertusvereniging vzw) in Flanders (Belgium) (Group B). No birds were killed for the purpose of this study. From this second group, the preen gland was removed and the first, third and fifth tail feather was pulled at both sides. Feathers were kept in paper envelopes at room temperature. Preen glands were stored at -20°C.

Feather analysis – Feathers from group A were all washed with distilled water (n=12). For group B, right feathers were washed with distilled water (n=6), acetone (n=6) or surfactants (n=4; 1% RBS 35/aceton (50:50)), while left feathers (n=16) were not washed and kept as controls. Feathers were dried at room temperature and cut in pieces of ~1 mm. 100-300 mg was weighed and incubated overnight at 40°C with HCl (4N) and a mixture of hexane/dichloromethane (4:1, *v:v*). After liquid extraction, clean-up was performed on acidified silica[8].

Serum analysis (Group A) – The procedure for the serum analysis was described by Covaci & Voorspoels[9]. Approximately 500 µl serum was spiked with internal standards (PCB 143 and BDE 77/128), mixed with 0.5 ml formic acid and 1 ml water and vortexed (20 min). The serum was extracted using SPE cartridges (OASIS™ HLB). Clean-up was done using columns filled with 0.5 g acid silica and 0.5 g Na_2SO_4.

Preen gland analysis (Group B) – A sample of approximately 1.5 g was mixed with anhydrous Na_2SO_4 and spiked with internal standards (PCB 143 and BDE 77/128). Further sample treatment and analysis were performed according to previously described methods[10]. Briefly, extraction was carried out with 100 ml hexane/acetone (3:1, v/v) in an automated Soxhlet extractor in hot extraction mode for 2.5 h. The lipid content was determined gravimetrically on an aliquot of the extract (1 h at 105 °C), while the rest of the extract was cleaned up on a column filled with 8 g acidified silica and eluted with 15 ml hexane and 10 ml dichloromethane. The eluate was concentrated to 100 ml under a gentle nitrogen stream and transferred to an injection vial.

GC/MS analysis - For PBDEs and DDTs, analysis was done using a GC/MS equipped with a HT-8 capillary column (25 m × 0.22 mm × 0.25 µm), operated in electron capture negative ionisation (ECNI) mode. PCBs were analysed using a GC/MS equipped with a DB-1 capillary column (30 m × 0.25 mm × 0.25 µm), operated in electron ionisation (EI) mode.

Statistical analysis - All statistical analyses were performed using SPSS 14.0 for Windows (SPSS Inc. 2005) and GraphPad Instat® version 3.06 for Windows (GraphPad Software Inc.). Samples with levels below the LOQ were assigned a value of p x LOQ, with 'p' the proportion of measurements with levels above the LOQ. Compounds with over 50% of the measurements below the LOQ were excluded from statistical analysis. Data were not normally distributed (Shapiro-Willks test, $p > 0.05$) even after log transformation. Therefore, non-parametric Spearman Rank correlations were calculated between levels in feathers and levels in serum or preen gland.

Results and Discussion

Table 1: Median (range) levels of different organic compounds in serum, preen gland and tail feathers of magpies. Values are expressed in ng/g ww or ng/ml for blood serum. '-' < LOQ

Compounds	Serum A (ng/ml) N = 11	Feathers A (ng/g) N = 12	Preen gland B (ng/g) N = 16	Feathers B-control (ng/g) N = 16
DDE	4.91 (0.93-11.6)	2.04 (0.79-12.9)	722 (61.0-4530)	24.6 (0.50-1670)
DDT	-	0.92 (0.38-4.48)	9.92 (2.28-294)	2.54 (0.14-18.1)
CB 52	-	4.35 (0.46-23.6)	-	-
CB 99	0.18 (0.08-10.4)	5.76 (0.46-15.8)	3.55 (1.46-280)	-
CB 101	-	33.0 (7.86-70.3)	0.83 (0.42-11.9)	0.51 (0.25-8.22)
CB 110	-	26.6 (8.48-66.3)	-	-
CB 118	-	15.4 (4.14-30.9)	2.19 (0.60-102)	0.31 (0.19-6.35)
CB 128	-	-	3.71 (1.61-346)	-
CB 138	1.20 (0.34-27.7)	11.6 (0.18-44.8)	11.2 (3.74-391)	1.33 (0.20-18.8)
CB 149	-	-	0.94 (0.00-9.90)	-
CB 153	3.61 (0.70-37.6)	10.9 (4.50-38.1)	33.0 (14.2-1250)	1.44 (0.20-23.2)
CB 156	0.09 (0.06-3.18)	0.49 (0.16-4.14)	1.60 (0.47-126)	-
CB 170	0.52 (0.09-4.73)	0.63 (0.30-9.66)	5.47 (2.37-176)	0.31 (0.14-4.24)
CB 180	1.04 (0.16-5.82)	1.09 (0.49-13.2)	10.2 (4.83-421)	0.55 (0.18-8.68)
CB 183	0.16 (0.07-1.18)	0.38 (0.13-2.14)	1.58 (0.68-77.2)	-
CB 187	0.68 (0.09-2.36)	0.83 (0.45-4.21)	7.51 (3.05-246)	0.34 (0.15-4.52)
CB 199	-		3.27 (1.22-483)	-
BDE 47	0.045 (0.013-0.32)	0.25 (0.09-1.02)	0.90 (0.13-3.03)	0.11 (0.07-0.95)
BDE 99	0.066 (0.009-0.31)	0.17 (0.08-1.08)	1.38 (0.39-22.3)	0.15 (0.07-1.78)
BDE100	-	-	0.43 (0.25-8.34)	-
BDE 153	-	-	0.89 (0.26-8.89)	-
BDE 154		-	0.26 (0.09-2.71)	-

Table 1 gives an overview of concentrations of organic compounds that were measured in the preen gland (tissue + oil), serum and tail feathers of magpies. HCB, OxC, TN and *p,p'*-DDD were only measured in the preen gland samples at median concentrations of 3.22 ng/g, 4.85 ng/g, 1.15 ng/g and 0.63 ng/g, respectively. A large variation in concentrations can be observed among tissues (Table 1). Maximum concentrations of most compounds were found highest in the preen gland samples, which is also the most lipid rich tissue. Concentrations in the blood serum were relatively low in comparison to the other two sampling methods. This could be due to the low amount of serum that could be obtained for individual birds. Most compounds could be measured in the feathers, with the exception of the PBDEs. Only BDE47 and BDE 99 could be quantified. In the feathers of predatory birds BDE 153 was detectable as well[1,2]. In Table 1 it can be observed that median concentrations of the lower chlorinated PCBs were somewhat higher in the feathers than in the preen gland, while the opposite was true for the higher chlorinated compounds.

Table 2 gives an overview of the correlations between levels in feathers and preen gland and feathers and serum. Correlations were found highest for levels in unwashed feathers and preen gland. This could indicate a potential route of external contamination with organic pollutants onto the feathers during preening. The large decrease in r_s for BDE 47 in washed feathers could be due to levels close to the LOD, and thus a higher uncertainty of the quantified data. Correlations between serum and feathers were found highest for the higher chlorinated PCBs.

Table 2: Spearman correlation coefficients (r_s) calculated for levels in tail feathers (ng/g) and blood serum (ng/ml) of magpies captured at the University of Antwerp (Belgium) (Group A; n=11), and for levels of organic compounds in tail feathers (ng/g) and preen gland (ng/g ww) of magpie cadavers from Belgium (Group B; n=16). The feathers of group A birds were all washed with distilled water. The right feathers of group B were washed with distilled water (n=6), acetone (n=6) or surfactants (n=4), while the left feathers were not washed (control).

Compounds	Feathers A – Serum A N = 11	Control feathers B – Preen gland B N = 16	Washed feathers B(*) – Preen gland B N = 16
DDE	0.31	0.82 ***	0.62 *
DDT	---	0.48	0.50 *
CB 99	-0.24	---	---
CB 101	---	0.31	0.42
CB 118	---	0.73 ***	0.90 ***
CB 138	0.32	0.78 ***	0.61 *
CB 153	0.16	0.89 ***	0.74 ***
CB 156	0.55	---	---
CB 170	0.88 ***	0.88 ***	0.74 ***
CB 180	0.75 **	0.89 ***	0.81 ***
CB 183	0.58	---	---
CB 187	0.63 *	0.90 ***	0.80 ***
BDE 47	0.59	0.57 **	0.10
BDE 99	0.40	0.62 **	0.66 **

(*) The feathers of the different washing procedures were pooled to increase the sample size.
* p<0.05, ** p<0.01, *** p<0.001

We have further investigated the relationship between the different tissues, by comparing their profiles. In Figure 1 the PCB profile is presented. Significant differences were found for congeners CB 138 and CB 153 between feathers and preen glands (Figure 1B). CB 138 has a higher contribution in the feathers, while the contribution of CB 153 is lower. The opposite is true for the preen glands. With regard to the PBDEs, BDE 47 has a higher contribution in the feathers, while BDE 99 has a higher contribution in the preen gland ($p < 0.001$). The only significant difference that could be found between the profile of feathers and blood serum is the contribution of CB 138 (Figure 1A). CB 138 contributes more to the PCB profile in feathers than in serum ($p < 0.01$). No differences in PBDE profile could be found between feathers and serum.

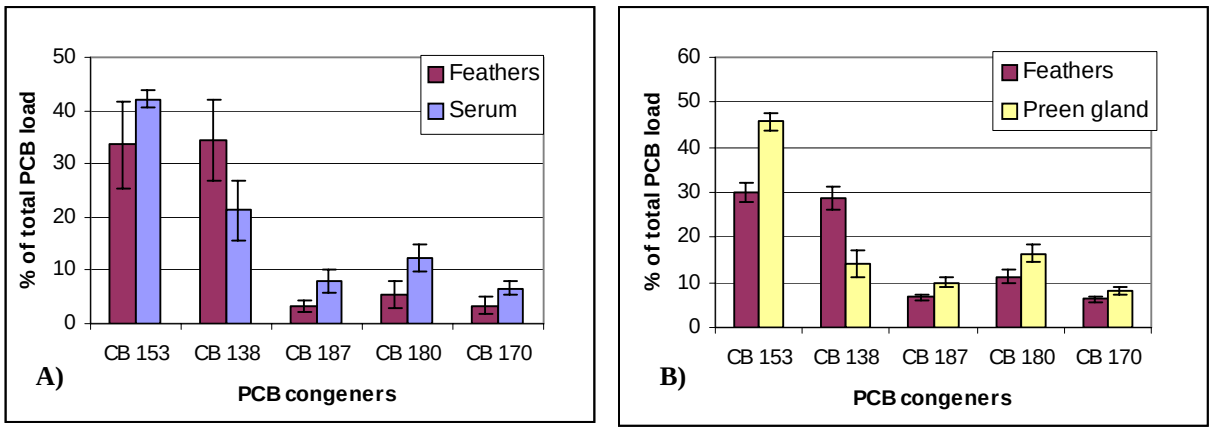

Figure 1: Profile of PCBs (% mean ± 2SE) in A) serum and tail feathers of magpies captured at the University of Antwerp (n=11), B) preen gland and unwashed tail feathers of magpie cadavers collected in Belgium (n=16).

In this paragraph, the different non-destructive biomonitoring methods will be evaluated. With regard to strict legislation on bird protection in the European Union, methods for non – destructive biomonitoring are necessary to be developed and evaluated. Although not included in this study, eggs have been successfully employed as a non – destructive biomonitoring method[11]. One egg can reflect the contamination of the whole clutch[11]. This method is not applicable to species which produce only a limited amount of eggs. Voorspoels et al.[12] have previously investigated levels in serum of predatory birds. Serum gives an idea of short term exposure and can be highly variable among seasons and individuals. Furthermore, the amount of blood that can be collected is limited (depending on the species) and therefore the quantification of organic pollutants may encounter some analytical problems. On the contrary, preen gland oil is lipid rich and may contain high concentrations of organic pollutants[13]. The quantification is therefore easier and more reliable. The collection of both blood and preen oil requires some expertise. In comparison the collection of feathers is relatively quick and easy. Major advantages of using feathers are the easy non-destructive sampling, simple storage and mailing[5]. Elaborate feather collections are available in some museums, which can be used to study time trends[5]. Even so, levels in feathers may be too low to quantify in small birds with a low trophic position[8].

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