# USEFULNESS OF BIRD FEATHERS AS A NON-DESTRUCTIVE BIOMONITOR FOR ORGANIC POLLUTANTS

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## Introduction

Birds have extensively been used in the past as biomonitors of environmental contamination with persistent organic pollutants[1,2], since they are situated high on the food chain and they are sensitive to environmental changes[3]. For practical and ethical reasons, methods for non-destructive biomonitoring have been developed[3]. The use of hair, a keratinous tissue, has recently been evaluated as a method for the analysis of persistent organic pollutants in mammals [4,5]. Since feathers are composed of a keratinous matrix as well, they are potentially useful to study contamination with organic pollutants. In contrast to hair, which is continuously growing, feathers grow only for a certain period of time and are connected to the blood stream (and its circulating pollutants) only during this limited time period.

Bird feathers have been used previously for monitoring heavy metals in several studies, but the use of feathers as monitors of organic pollutants has only recently been investigated[6,7]. Dauwe et al.[6] analyzed organic pollutants in feathers of a small passerine bird (*Parus major*), but they could not measure levels of PBDEs and some OCPs above LOQ. Moreover, given the large amount of feathers needed for analysis, feather sampling could not be considered non-destructive for passerine birds. Therefore the study of Jaspers et al.[7] was performed on a predatory bird species, the common buzzard (*Buteo buteo*), with a high position on the food chain. One tail feather of a buzzard was found to be sufficient to quantify concentrations of most PCBs, PBDEs and DDTs. Furthermore, positive correlations were found between levels of organic pollutants in feathers and muscle or liver tissue. Therefore, feathers of common buzzards, and probably other predatory birds, can be useful as a non-destructive biomonitor for contamination with organic pollutants[7].

Here we present the results of a compilation of studies measuring concentrations of organic pollutants in feathers of different bird species. We also investigate if there is a correlation between levels in liver or muscle and levels in the corresponding feathers of each species, and if combined in a meta-analysis to examine the overall pattern.

## Materials and Methods

108 carrions of 8 different bird species (Aquatic: 9 grey herons - *Ardea cinerea*, 12 herring gulls - *Larus argentatus*, 11 common moorhens - *Gallinula chloropus*; Terrestrial: 9 barn owls - *Tyto alba*, 10 long-eared owls - *Asio otus*, 43 common buzzards – *Buteo buteo*, 3 kestrels - *Falco tinnunculus*, 11 sparrowhawks - *Accipiter nisus*) were collected in collaboration with Wildlife Rescue Centres (WRCs) in Flanders. The birds were found dead or had died shortly after collection. Frequent causes of death included traffic accidents, natural causes and starvation. No birds were killed for the purpose of this study. We collected the outermost tail feathers (and primary wing feathers for the buzzards) and stored them in paper envelopes until further analysis. Feathers were washed with distilled water, dried at room temperature and cut in pieces of ~1 mm. Depending on the species, 100 - 500 mg of the feathers was weighed and incubated overnight at 40°C with hydrochloric acid (4 N) and a mixture of hexane and dichloromethane (4:1, *v:v*). After liquid extraction, clean-up was performed on acidified silica, as described by Dauwe et al.[6]. Liver and pectoral muscle were excised and stored at -20°C until sample preparation. The results of the tissue analysis are reported elsewhere[2, 8].

In all samples, 7 PBDE congeners (28, 47, 99, 100, 153, 154, and 183), brominated biphenyl (BB) 153, 25 PCB congeners, and p,p'-DDT and metabolites (p,p'-DDE and p,p'-DDD), expressed here as DDTs, were analysed.

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.

All statistical analyses were performed using Statistica for Windows (Statsoft 1997), GraphPad Instat® version 3.06 for Windows (GraphPad Software Inc.) and Comprehensive Meta-Analysis version 1.025 (Biostat® 1998). 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 (i.e. CBs 18, 28, 52, 74, 105, 132, 156 and 167 and *p,p'*-DDD). Data were not normally distributed (Shapiro-Willks test, $p > 0.05$) and were therefore log-transformed (log10 (x+1)). Parametric Pearson correlations were calculated between log-transformed concentrations of organic pollutants in feathers and levels in liver / muscle tissue. We performed a meta-analysis to provide estimates of the true effect sizes for the general relationship between levels of organic pollutants in feathers and levels in internal tissues, based on the calculated Pearson correlation coefficients of the different bird species. Meta-analytical techniques provide constructive methods to summarize data by examining the generality of a relationship, taking sample size into account[9]. Analyses were performed together for all birds and separately for the aquatic and the terrestrial species.

**Results and discussion**

1. Levels and profiles in feathers

All investigated classes of organic pollutants could be measured in feathers of the bird species under study, except for the common moorhen that had non-detectable levels for PBDEs. Sparrowhawks showed high levels of all analysed organic pollutants. Herring gulls showed highest PCBs levels (median 208 ng/g), while DDTs levels in feathers were rather low (median 9.1 ng/g) in comparison to other predatory bird species (up to 473 ng/g in sparrowhawks). Except for the common moorhen, PBDEs were quantified in all samples with levels ranging from 0.33 to 53 ng/g feather. Overall, levels of Σ PBDEs were 5 to 50 times lower than levels of Σ PCBs in feathers. Concentrations measured in feathers were 100 to 1,000 times lower than levels reported in liver and muscle tissue of these birds[2, 8].

Because common moorhens feed on a diet with varying proportions of animal and plant material[10], they had lower intake of organic pollutants and therefore showed lower accumulation compared to predatory birds. On the other hand, the diet of sparrowhawks consists almost entirely of small birds (up to 98%). The other terrestrial predatory birds analysed in the present study mainly feed on small mammals and to a lesser extent small birds[10]. Small bird species feeding on seeds treated with seed dressings can accumulate pesticides, which could result in higher concentrations of pesticides in the predatory birds that preyed on them[1]. This could explain the relatively high levels of organic contamination in sparrowhawks and the differences in accumulation compared to the other terrestrial predatory birds. The different accumulation profiles of herring gulls can possibly be explained by its very opportunistic feeding habits, consuming not only fish, but a variety of items, even garbage[10].

Overall, concentrations measured in the predatory birds of this study were higher than concentrations measured by Dauwe et al.[6]. Moreover, PCBs, DDTs as well as PBDEs could be quantified in one single feather of the predatory birds under study. Therefore feathers of predatory birds seem more suitable as an assessment tool for environmental levels of organic pollutants, than feathers of small passerines (see above) or common moorhens.

A different PBDE and PCB profile could be observed between feathers and muscle tissue in the predatory birds under study. BDE 47 was the most prominent congener in feathers of most bird species, while this congener had a lesser contribution in muscle tissue, for which BDE 99 and 153 were more important in most bird species. BDE 47 had the highest contribution in the aquatic species (i.e. heron and gull), in feathers as well as in muscles, while the contribution of BDE 99 and 153 increased towards the terrestrial species. Concentrations of BDE 183 in heron were low, but concentrations in herring gull were similar to levels in terrestrial birds of prey. Non-

persistent chlorinated PCB congeners (i.e. CB 95, 101 and 110) had a relatively higher contribution in feathers than in muscle tissue. Contributions of these congeners were particularly high in feathers of the common moorhen. Earlier research has also indicated that the percentage of lower chlorinated congeners (tetra- and penta-CB congeners), including non-persistent congeners, measured in feathers is higher than in internal tissues[6].

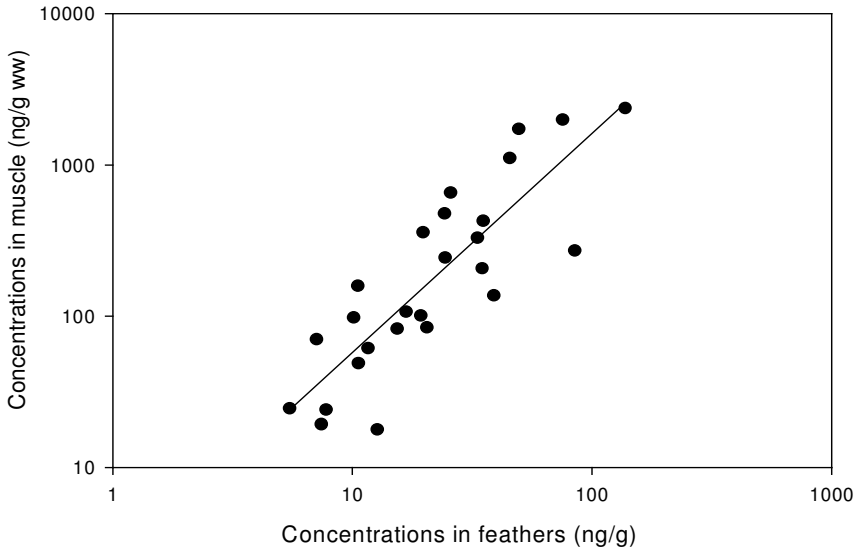
The different PBDE and PCB profiles in feathers and muscle may possibly be explained by external contamination of organic compounds on the feather surface. Although, the importance of external contamination has been extensively studied in the area of heavy metal monitoring via feathers[11,12], external contamination onto the feather surface should also be considered when measuring concentrations of organic pollutants. Lower brominated PBDE / chlorinated PCB congeners are assumed to contribute more to external contamination than higher brominated / chlorinated congeners, similar to what is observed by Dauwe et al.[6] for PCBs in feathers.

2. Correlations between feathers and tissues

For PCBs ($0.21 < r < 0.89$) and PBDEs ($0.12 < r < 0.75$), correlations between feather and muscle were significant in three of the seven bird species, while for *p,p'*-DDE ($0.17 < r < 0.86$) significant correlations were established in five of the seven bird species. When correlations were recalculated excluding the birds that had died due to starvation, correlation coefficients for the terrestrial birds were found higher (up to $r = 0.92$ for *p,p'*-DDE), indicating the importance to control for the condition of the birds. Similar correlation coefficients as those in the present study have been reported for heavy metals between levels in feathers and internal tissues ($0.51 < r < 0.84$ between feathers and liver, $0.31 < r < 0.74$ between feathers and muscle)[12]. d'Havé et al.[5] recently observed positive relationships between hair and internal tissues that, after removal of two outliers in their dataset, were in the lower range ($0.43 < r < 0.53$, $n = 40$) of correlations found in our study.

A high variability could be seen among species in both correlation coefficients and significance. Only for the buzzard most correlations were found significant. For the aquatic species grey heron and herring gull, correlation coefficients were found low and not significant. However, for the common moorhen, also an aquatic bird species, a high and significant correlation was found for levels of Σ PCBs between feather and muscle, in addition to a significant correlation in p,p'-DDE levels. This discrepancy might be explained by variation in diet: herring gulls are very opportunistic feeders, changing their diet in accordance to available items (also garbage), while common moorhens feed on a more controlled diet of insects and plants[10].

**Figure 1**: Relationship between levels of PCBs in feathers and muscle tissue of common buzzards (n=26) from Belgium.



Results for the meta-analyses are presented in Table 1. We performed meta-analyses with all birds combined and for the aquatic and terrestrial species separately. Almost all correlations were found significant, with the exception of some correlations for the aquatic species (mostly with liver). Again, when the birds killed by starvation were excluded, correlation coefficients of the terrestrial species improved (Table 1B).

**Table 1:** Results of the meta-analysis performed on Pearson correlation coefficients of $\log_{10}$ concentrations between feathers (ng/g) and muscle or liver tissue (ng/g ww) of different predatory birds from Belgium. A) Including all birds, B) Excluding birds which have died by starvation. * $p < 0.05$, ** $p < 0.01$

| Meta-analysis | Tissue | A) | N | Σ PCBs | Σ BDEs | *p,p'*-DDE | B) | N | Σ PCBs | Σ BDEs | *p,p'*-DDE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| aquatic | Muscle | | 32 | 0.62 ** | 0.47 * | 0.48 ** | | 22 | 0.65 ** | 0.36 | 0.36 |
| | Liver | | 9 | 0.21 | 0.12 | 0.17 | | 4 | 0.05 | -0.22 | 0.57 |
| Terrestrial | Muscle | | 73 | 0.71 ** | 0.70 ** | 0.59 ** | | 36 | 0.83 ** | 0.76 ** | 0.59 ** |
| | Liver | | 73 | 0.57 ** | 0.47 ** | 0.40 ** | | 36 | 0.64 ** | 0.52 ** | 0.45 ** |
| Combined | Muscle | | 105 | 0.69 ** | 0.66 ** | 0.56 ** | | 58 | 0.79 ** | 0.71 ** | 0.52 ** |
| | Liver | | 82 | 0.55 ** | 0.44 ** | 0.38 ** | | 40 | 0.63 ** | 0.50 ** | 0.45 ** |

In general, higher correlation coefficients were found between feathers and muscle samples than between feathers and liver samples in all species. Assuming that concentrations in feathers reflect the circulating concentrations in the body at the time of their formation, feathers may not reflect recent changes in contamination. Since the turnover rate in liver (a highly metabolically active tissue) is higher than in muscle, concentrations in liver probably reflect more recent exposure, which may explain the lower correlation coefficients found between feather and liver samples.

Although, at present, our results cannot explain all variation observed between levels in feathers and internal tissues, predatory bird feathers can provide an overall assessment of contamination levels. Given that feathers can be easily preserved and that bird collections in museums and private collections date from the late 1700's, feathers may be useful for retrospective biomonitoring of organohalogenated pollutants, as successfully done for heavy metals[12], and to study regional and temporal trends of contamination with organohalogens.

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