PERSISTENT ORGANIC POLLUTANTS IN FEATHERS AND BLOOD FROM NESTLING RAPTORS OF NORTHERN NORWAY: DIFFERENCES AMONG SPECIES AND RELATION TO STABLE ISOTOPES

Jaspers V L B[1], Eulaers I[1], Covaci A[2], Herzke D[3], Schnug L[4], Johnsen T[5], Halley DJ[6], Eens M[1], Bustnes J O[5]

[1]Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium; [2] Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium; [3]Norwegian Institute for Air Research (NILU), Polar Environmental Centre, Polarmiljøsenteret, 9296 Tromsø, Norway; [4]Norwegian Institute for Agricultural and Environmental Research, Soil and Environment Division, Fr. A. Dahlsvei 20, N-1432 Ås, Norway; [5]Norwegian Institute for Nature Research (NINA), Polar Environmental Centre, Polarmiljøsenteret, 9296 Tromsø, Norway; [6]Norwegian Institute for Nature Research (NINA), 7485 Trondheim, Norway

Abstract

As part of an ongoing project to assess the impact of organic pollutants and biological stressors on the fitness and survival of avian top predators in different northern ecosystems, we collected body feathers and blood samples from nestlings of three different raptor species. Here we investigate the concentrations and profiles of POPs in blood and feathers, the relationships between them and their relation to trophic position (determined by stable isotopes) and ecology.

We found that concentrations of most POPs could be quantified in feathers from nestlings, with *p,p'*-DDE and sum PCBs as the most important compounds. Concentrations in feathers were significantly related to concentrations in blood in most cases. Furthermore, concentrations of POPs in feathers could also be related to concentrations of stable isotopes in the feathers, except for the golden eagle (*Aquila chrysaetos*). Further research is necessary to conclude on the usefulness of nestling raptor feathers for biomonitoring purposes.

Introduction

In 2008 an international project (RAPTOR-2015) funded by the Norwegian Research Council, was set up to investigate the impact of organic pollutants and other environmental and biological stressors on avian top predators in different northern ecosystems and to assess their potential vulnerability to environmental changes. Northern ecosystems are experiencing increasing stress from both climate changes and organic pollutants[1]. Recent evidence suggests that a combination of pollutants and other stressors may have strong adverse effects in biota[2], and that even low levels of pollutants may be harmful for wildlife. Future environmental changes are expected to further increase stress on northern populations by altering a wide range of natural stressors[1].

In order to assess the vulnerability of northern ecosystems to environmental/climate change, it is imperative to investigate the current stress posed by organic pollutants. Since most organic pollutants biomagnify through the food chain, avian top predators are very interesting species to monitor[4]. However, these species are often protected, and samples (e.g. tissues, blood, and eggs) are difficult to obtain. Recently, feathers have been proven to be useful as non-destructive and non-invasive biomonitors for organic pollutants[5-8]. Feathers can be obtained from living birds causing minimal harm. Another possibility is to collect moulted feathers at the nest. Furthermore, feathers are easily stored and transported.

In the present study we investigate whether the concentrations of organic pollutants measured in feathers sampled from nestling raptors at the nest, reflect the concentrations in their blood. Furthermore, concentrations of organic pollutants will be related to trophic position (determined by stable isotope measurements in feathers) and to specific ecosystems (coast versus inland) in which the bird species reside.

Materials and Methods

At the end of June and the beginning of July 2008, body feathers were collected from nestlings of three raptorial species in Troms and Finnmark Counties, Norway. The three raptor species included in this study were: the golden eagle (*Aquila chrysaetos*) for the mountainous ecosystem, the goshawk (*Accipiter gentilis*) for the woodland ecosystem, and the white-tailed sea eagle (*Haliaeetus albicilla*) for the coastal ecosystem. From each

bird, five feathers were pulled from both the chest and the back, and the central tail feather was cut off from some goshawks as well. All feathers were sent in envelopes by mail to the Toxicological Center at the University of Antwerp (Belgium) for analysis.

Feathers were washed with distilled water, dried at room temperature and cut in pieces of ~1 mm. An amount between 50 and 800 mg feather 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[6]. In all samples, 39 polychlorinated biphenyl (PCB) congeners (CB 18, 28, 31, 44, 47, 49, 52, 74, 87, 95, 99, 101, 105, 110, 118, 128, 132, 138, 146, 149, 151, 153, 156, 158, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 196/203, 199, 205, 206 and 209), 8 polybrominated diphenyl ether (PBDE) congeners (BDE 28, 47, 49, 99, 100, 153, 154 and 183), dichlorodiphenyltrichloroethane (*p,p'*-DDT and *o,p'*-DDT) and metabolites (*p,p'*-DDE *p,p'*-DDD, *o,p'*-DDE and *o,p'*-DDD), hexachlorocyclohexanes (HCHs; α-, β- and γ-HCH), chlordanes (CHLs; *cis*-chlordane (CC), *trans*-chlordane (TC), *trans*-nonachlor (TN) and oxychlordane (OxC)) and hexachlorobenzene (HCB) were analysed. For PBDEs, analysis was done using a GC/MS equipped with a DB-5 capillary column (30 m × 0.25 mm × 0.25 µm), operated in electron capture negative ionisation (ECNI) mode. PCBs and DDTs were analysed using a GC/MS equipped with a HT-8 capillary column (25 m × 0.22 mm × 0.25 µm), operated in electron ionisation (EI) mode.

Analyses of POPs in blood plasma were performed by Dr. Dorte Herzke and Lisbeth Schnug in the laboratory of the Norwegian institute of Air Research (NILU) in Tromsø (Norway). A detailed description of the procedure is given by Bustnes et al.[9]. Bloodplasma was analysed for 15 PCB-congeners (18, 28, 52, 99, 101, 105, 118, 128, 138, 149, 153, 180, 183, 187, 194), 6 PBDE-congeners (28, 47, 99, 100, 153, 154), HCB, OxC, TN, CN, TC, CC, α-, β- and γ-HCH, *p,p'*- and *o,p'*-DDT, *p,p'*- and *o,p'*-DDE. Concentrations of pollutants are expressed in pg mL^{-1}.

Stable isotope measurements were carried out by Prof. Dr. Masao Minagawa and Aiko Agui in the laboratory of Integrated Environmental Science at the University of Hokkaido (Japan). Nestling feathers (N_{total} = 41) were analysed for ^{13}C and ^{15}N isotopes with a mass spectrometer (Finnegan MAT 252) coupled by a Conflow II interface to a Element Analyzer N1500. Ratios of ^{13}C and ^{15}N isotopes are expressed as δ in ‰ according to the formula $\delta X = ((R_{sample} - R_{standard}) / R_{standard}) * 1000$, with X as ^{13}C of ^{15}N and R as the corresponding ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the sample or standard.

All statistical analyses were performed using SAS 9.2 for Windows (SAS Institute Inc.) and XLStat 2009.3.01 for Windows (Addinsoft TM). 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 and were therefore logtransformed. The logtransformed data met the assumptions of normality and consequently parametric tests were performed. Pearson correlations were performed to investigate the relationships between concentrations of POPs in blood and feathers of nestling raptors and between concentrations of POP and stable isotopes measured in the feathers. Furthermore, two way ANOVAs were performed to investigate the differences in concentrations among species in blood and feathers respectively.

Results and Discussion

Levels

The most important compounds could be quantified in the feathers of all nestlings. As shown in Table 1. *p,p'*-DDE and sum PCBs were the most important compounds that could be measured in the feathers of the goshawk and the golden eagle. However, *p,p'*-DDE was found at much lower concentrations in the sea eagle ($F = 4.6$; $p < 0.05$). Overall, sum PBDEs were less than 10% of sum PCBs, while HCB and *β*-HCH were found at much lower concentrations (20% and 10% of sum PBDEs, respectively). In feathers from nestling raptors CHLs were not quantifiable in more than 50% of the cases.

Table 1: Mean concentrations ± SE (ng/g feather) of organic pollutants in feathers of avian top predators from northern ecosystems. * Sum PCBs includes: CB 99, 118, 138, 153, 170, 180, 183 & 187; ** Sum PBDEs includes: BDE 47, 49, 99 & 100

ng/g feather	Goshawk (GH) (n = 18)	Sea Eagle (SE) (n =5)	Golden Eagle (GE) (n =15)
Sum PCBs*	39.7 ± 10.1	34.8 ± 15.5	32.3 ± 6.7
Sum PBDEs**	3.54 ± 0.51	2.21 ± 0.68	1.18 ± 0.26
p,p'-DDE	43.9 ± 9.8	8.30 ± 3.41	33.3 ± 8.1
HCB	0.90 ± 0.21	0.60 ± 0.09	0.54 ± 0.10
β-HCH	0.30 ± 0.07	0.20 ± 0.03	0.29 ± 0.08

In comparison, the levels in blood are given in Table 2. For some individuals, the blood sample was too small to perform POPs analysis, which is why the number of samples is different for feathers and blood. For the profiles and correlation analysis, we only made use of the individuals for which both blood and feathers were analysed.

Table 2: Mean concentrations ± SE (pg/mL) of organic pollutants in blood plasma of avian top predators from northern ecosystems. * Sum PCBs includes: CB 99, 118, 138, 153, 180 & 187; ** Sum PBDEs includes: BDE 28, 47, 99 & 100; # In GE only TN and CN were quantifiable > 50%

pg/ml plasma	Goshawk (GH) (n = 20)	Sea Eagle (SE) (n = 5)	Golden Eagle (GE) (n =7)
Sum PCBs*	10900 ± 2400	28500 ± 10900	12300 ± 3800
Sum PBDEs**	960 ± 270	1500 ± 670	420 ± 170
p,p'-DDE	8700 ± 1400	5800 ± 2100	10800 ± 9400
HCB	580 ± 120	760 ± 130	460 ± 180
Sum CHLs	910 ± 170	2400 ± 770	200 ± 94[#]

Again, *p,p'*-DDE and sum PCBs were the most prominent compounds in goshawk and golden eagle, whereas *p,p'*-DDE was only 25% of the concentrations of sum PCBs in sea eagle blood. This agreement suggests that concentrations in feathers may reflect the contamination in the blood. Furthermore, sum PBDEs and HCB were found at much lower concentrations, while β-HCH was quantifiable in the blood of the GE only. In sea eagle BDE 153 and 154 were quantifiable in addition to BDE 28, 47, 99 and 100. BDE 154 was also measured in 14 samples of the GH, but not in the GE.

Profiles

The profiles of PCBs (Figure 1) and PBDEs (Figure 2) were compared between feathers and blood for the different species. Only congeners that could be quantified in both blood and feathers are included.

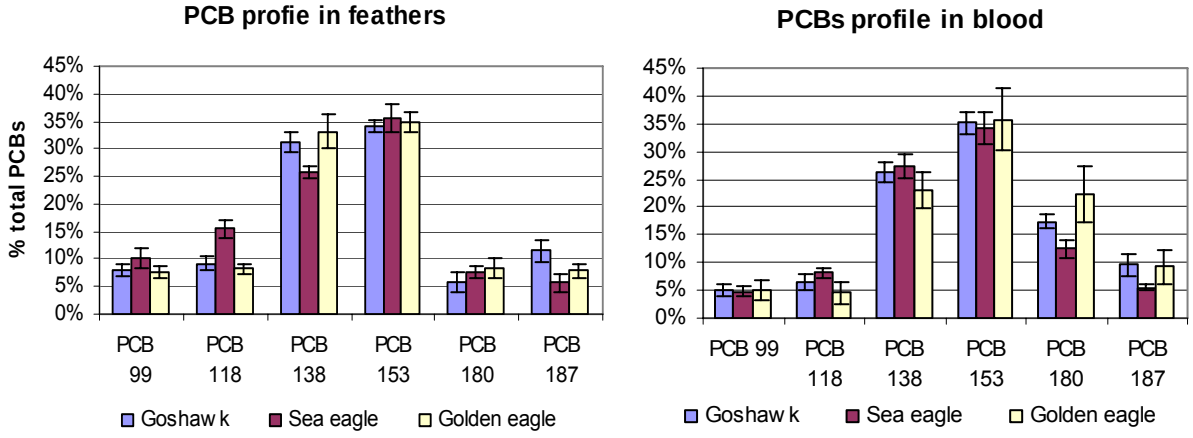

Figure 1: PCBs profile (mean% ± 2SE) in feathers and blood of nestling raptors from Northern Norway.

Overall, the profile in blood is reflected by the profile in the feathers, which indicates that nestling feathers may be useful for biomonitoring purposes. CB 138 and CB 153 are the most important congeners in all species and in both matrices. However, the higher chlorinated congener CB 180 is more important in blood in comparison to feathers, in which CB 99 and CB 118 and also CB 138 have a slightly higher contribution. The abundance of higher chlorinated PCBs in feathers has been observed before[7,8].

The PBDE profile is also roughly comparable between blood and feathers, except for the goshawk. In the goshawk, BDE 100 is only about 15% in feathers, while this congener has a contribution to the profile in blood of about 40%. BDE 47 is the most prominent congener in the sea eagle both in blood and feathers, which can be explained by its aquatic habitat[10,11].

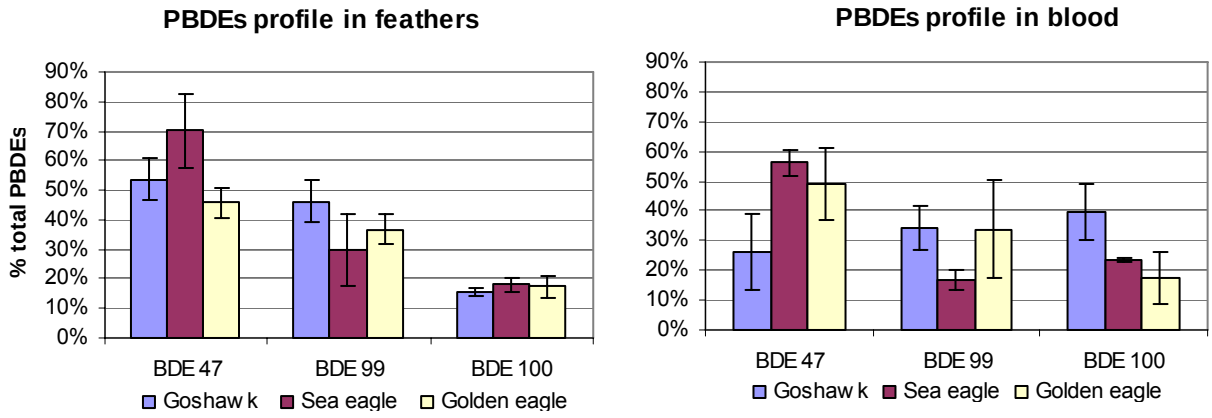

Figure 2: PBDEs profile (mean% ± 2SE) in feathers and blood of nestling raptors from Northern Norway.

Correlations between feathers and blood

Previous studies have shown that concentrations in feathers are significantly related to concentrations in internal tissues, blood and preen oil[5-8,12]. To study the relationship between newly formed feathers and the levels in the blood during feather growth, we performed correlation analysis for each species. Significant correlations between levels of POPs in blood and feathers of the goshawk were found for CB 99, CB 118, CB 138, CB 153, CB 180, CB 187, sum PCBs, DDE and BDE 47 ($0.62 < r < 0.86$; $p < 0.05$; $n = 17$). This means that for the goshawk all commonly detected PCB congeners in blood and feathers were significantly related to each other. When the mean was calculated for the individuals per nest, all correlations mentioned above remained significant and even improved ($0.81 < r < 0.97$; $p < 0.05$; $n = 7$), with BDE 100 and sum PBDEs being significantly correlated between feathers and blood as well. In the golden eagle significant correlations were found for CB 99, CB 118, CB 138, CB 153, CB 180, CB 187, sum PCBs, BDE 47, BDE 100 and sum PBDEs ($0.77 < r < 0.93$; $p < 0.05$; $n = 7$). For the sea eagle significant correlations were found between levels in blood and feathers for CB 99, CB 118, CB 138, sum PCBs, BDE 47 and sum PBDEs ($0.88 < r < 0.91$; $p < 0.05$; $n = 5$), while CB 153, 180, 187 and BDE 100 had a p-value between 0.05 and 0.07. Considering that the sample size was very low ($n = 5$), increasing the sample size will probably lead to even more significant correlations between levels in blood and feathers for the sea eagle. All sea eagle samples were from separate nests.

Correlation to stable isotopes

For the goshawk $\delta^{15}N$ measured in feathers was significantly related to CB 99, CB 118, CB 138, CB 153, CB 170, CB 180, CB 183, CB 187, sum PCBs, DDE, BDE 47, BDE 99, BDE 100 and sum PBDEs in feathers ($0.52 < r < 0.83$; $p < 0.05$; $n = 18$). As shown in Figure 3, $\delta^{15}N$ values are highly variable in the goshawks. This is caused by the variability of food items in their diet[13], which may in turn have an influence on the accumulated POPs levels. On the other hand, no correlations were found for the levels of POPs in feathers with $\delta^{13}C$ in the goshawk, which can be explained by the fact that C^{13} fractionates only very weakly with trophic level, and is present at higher levels in the marine food chain in comparison to the terrestrial food chain. When the correlations were performed per nest, results remained the same, except for a non-significant relation between

$\delta^{15}N$ and BDE 99 and *p,p'*-DDE. For the sea eagle $\delta^{15}N$ was only significantly correlated with β-HCH ($r = 0.92$; $p < 0.05$, $n = 5$) and almost significantly with CB 153, CB 183 and BDE 100 ($0.84 < r < 0.87$; $0.054 < p < 0.073$; $n = 5$). In contrast, many significant correlations were found in this species with $\delta^{13}C$: for CB 99, CB 118, CB 138, CB 153, CB 180, CB 183, sum PCBs, HCB, *p,p'*-DDE and BDE 47 ($0.88 < r < 0.94$; $p < 0.05$; $n = 5$). $\delta^{13}C$ values indicate the contribution of food from marine origin to the diet. Since the sea eagle is not only feeding on fish, but also takes food from the shore, such as carrion, birds and small mammals[13], this will be reflected in the isotopic signature and will have an influence on the contamination with POPs. Because the dietary items are representing different food chains, this also obscures a clear relation with δ15N, which is variable among species. For the golden eagle, no correlations with $\delta^{13}C$ or $\delta^{15}N$ were found. The reason for this is not clear, but may possibly be explained by their diet, which is mainly consisting of different herbivores[14] varying slightly in $\delta^{15}N$, but not much in their POPs content as they all basically have the same diet.

Species differences

No significant differences were found among species for sum PCBs, individual PCBs congeners, HCB or β-HCH in feathers of nestling raptors. Average levels of sum PCBs in feathers of goshawk and sea eagle nestlings were 3-4 times lower compared to concentrations found in adult feathers of these species collected between 1999-2005 (pilot study presented at the Dioxin 2008)[15]. It is possible that the differences among species are more distinct in feathers from adult birds which accumulate higher concentrations. However, these adult feathers were collected in different years and from different nests. Concentrations in feathers from nestlings are better related to concentrations in feathers from their parents. In contrast to PCBs, very significant differences were found between the concentrations in feathers of the nestling goshawks and golden eagles for sum PBDEs, BDE 47, BDE 99 and BDE 100 and between the nestling goshawks and sea eagles for DDE (Table 1).

In blood, significant differences were found between the sea eagle and golden eagle for BDE 100 and between the goshawk and the golden eagle / sea eagle for BDE 47, which is also reflected in the profile (Figure 2). No significant differences were found for sum PBDEs, but a significant difference was found for sum PCBs between sea eagle and goshawk, and between goshawk and golden eagle for DDE. Sum HCHs were significantly different among the three species.

Besides differences in contamination levels, differences were also found in the stable isotopic signatures of these species, as shown in Figure 3. The sea eagle has higher $\delta^{15}N$ values, which means it is feeding higher up the food chain. This is reflected by the higher concentrations of sum PCBs, sum PBDEs and sum HCHs (Table 2). In feathers, very few significant differences among the species were found as mentioned above. This may be related to the lower concentrations found in feathers from nestlings compared to adults.

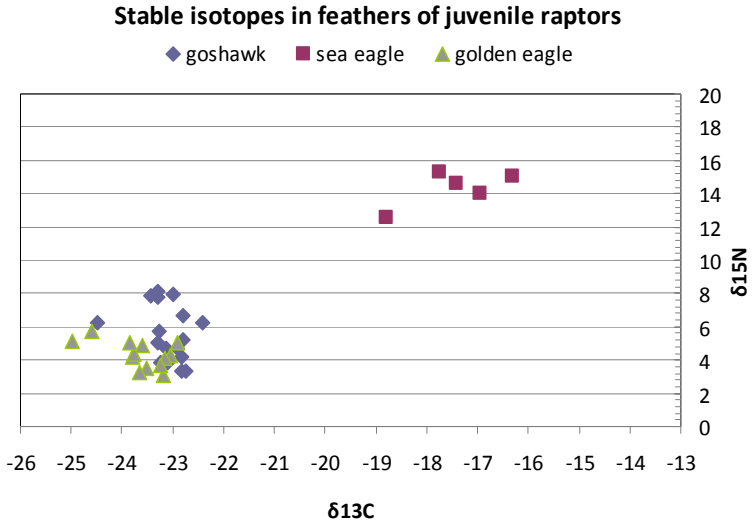

Figure 3: Stable isotope signatures in feathers of nestling raptors from Northern Norway.

Conclusions

Overall, the early results from the RAPTOR project suggest that feathers collected from nestlings at the nest may be useful to measure concentrations of organic pollutants in avian top predators from northern ecosystems. Furthermore, the concentrations and profiles in the feathers seem to reflect differences among species, although the concentrations were mostly not significantly different. Larger sample sizes, especially for the sea eagle, are necessary to study differences among species and habitats in more detail. Concentrations of POPs in feathers could also be related to concentrations of stable isotopes in the feathers, except for the golden eagle. Future research should focus on the relation of concentrations measured in nestlings with the concentrations found in their parents. More research is warranted to assess the usefulness of feathers of juvenile predatory birds for biomonitoring purposes.

Acknowledgements

Dr. Masao Minagawa and Aiko Agui are gratefully acknowledged for the analysis of the stable isotopes in feathers. Dr. Veerle Jaspers and Dr. Adrian Covaci are postdoctoral fellows at the Funds for Scientific Research Flanders (FWO). Prof. Dr. Eens is funded by the University of Antwerp and FWO. Dr. Jan Ove Bustnes is funded by NFR Project: ES421785: Persistent organic pollutants and natural stress in avian top predators of northern ecosystems; potential vulnerability to environmental change.

References

1. Sih A., Bell A. M. and Kerby J. L. *Trends Ecol. Evol.* 2004; 19: 274.
2. Bustnes J. O. , Erikstad K. E., Hanssen S. A., Folstad I. and Skaare J. U. *Proc. R. Soc. B* 2006; 273: 3117.
3. Boonstra R. *Integr. Comp. Biol.* 2004; 44: 95.
4. Jaspers V. L. B., Covaci A., Voorspoels S., Dauwe T. and Eens M., Schepens P. *Environ. Pollut.* 2006; 139: 340.
5. Jaspers V. L. B., Voorspoels S., Covaci A. and Eens M. *Biol. Lett.* 2006; 5: 283.
6. Jaspers V. L. B., Voorspoels S., Covaci A., Lepoint G. and Eens M. *Environ. Int.* 2007; 33: 328.
7. Jaspers V. L. B., Van den Steen E., Covaci A., Eens M. *Environ. Int.* 2007; 33: 766.
8. Jaspers V. L. B., Covaci A., Deleu P., Neels H. and Eens M. *Environ. Int.* 2008; 34: 741.
9. Bustnes J. O., Borga K., Erikstad K. E., Lorentsen S. H. and Herzke D. *Environ. Toxicol. Chem.* 2008; 27: 1383.
10. Bustnes J. O., Erikstad K. E., Bakken V., Mehlum F. and Skaare J. U. *Ecotoxicology* 2000; 9: 179.
11. Voorspoels S., Covaci A., Jaspers V. L. B., Neels H. and Schepens P. *Environ. Sci. Technol.* 2007; 41: 411.
12. Van den Steen E., Covaci A., Jaspers V. L. B., Dauwe T., Voorspoels S., Eens M. and Pinxten R. *Environ. Int.* 2007; 33: 257.
13. Cramp, S. and Simmons, K. E. L. 1983. Handbook of the birds of Europe the Middle East and North Africa. The birds of the Western Palaearctic. Vol III. Oxford University Press, Oxford.
14. Watson, I. 1997. The Golden Eagle. T and A.D. Poyser, London, UK.
15. Jaspers V. L. B., Covaci A., Johnsen Trond V., Eens M., Bustnes J. O. *Organohalogen Compounds* 2008; 70: 2364.