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Levels of PCBs in wild bird eggs: Considering toxicity through enzyme induction potential and molecular structure

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HIGHLIGHTS

- The metabolic potential of the PCBs showed agreement with biodegradability.
- Phenobarbital-type inducer and metabolic group I PCBs were prevalent.
- 88% of PCB congeners measured was found in more than 80% of samples.
- PCB levels, including dioxin-like PCBs, were highest in piscivore species.
- The high prevalence of CB-138, -153 and -180 make them ideal for monitoring PCBs.

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ABSTRACT

Polychlorinated biphenyls (PCBs) were analysed in wild bird eggs from industrialised areas in South Africa. The concentration, congener profile, feeding guild, potential associated risk and biology were investigated. PCBs were detected in all eggs with 30 congeners present in more than 80% of the samples. Σ_{34}PCB concentrations ranged between 0.9 and 296 ng g^{-1} wet weight (ww). The metabolic potential of the PCB metabolic groups showed good agreement with the biodegradability of the individual congeners. Phenobarbital-type (PB-type) inducer PCBs were prevalent, indicating the predominance of less toxic PCB congeners. However, non-*ortho* PCBs which were not included in the current analyses, could affect the toxic potential of the PCBs in the eggs requiring more investigation. Although the current levels of PCBs measured do not indicate a health risk to the birds assessed, the presence of mono-*ortho* PCBs at appreciable levels motivates for the assessment of dioxin-like chemicals in wild bird eggs.

1. Introduction

Polychlorinated biphenyls (PCBs), apart from some natural sources, entered the environment from extensive use in industrial applications. However, due to the hydrophobicity and biological recalcitrance of PCBs concern over the possible adverse health effects and long-range transport led to restricted use and eventual banning during the 1970s (Takasuga et al., 2006). Although, PCBs are banned or controlled on a global and regional scale they are still formed as unintended industrial by-products. Additionally, legacy sources of PCBs are still present and can contribute to the current PCB loading into the environment through leaching and re-volatilisation processes (UNEP Chemicals, 1999).

PCBs exhibit congener-specific toxicity where the structural specificity of PCBs for enzyme induction is a well-recognised indicator of potential toxicity (McFarland and Clarke, 1989). The presence of vicinal H-atoms in the *meta–para* position increases the susceptibility of the PCB-molecule to metabolism through enzymatic activity of the P450 system (Warner et al., 2005).

A group of microsomal cytochrome P450-dependent enzyme systems, known as mixed-function oxidase (MFO), is responsible for catalysing biotransformation processes of xenobiotics (McFarland and Clarke, 1989; Denison and Whitlock, 1995). The presence and activity of the cytochrome P450-enzymes determines an organism's ability to metabolise compounds such as PCBs and therefore influence the occurrence of these chemicals in biological tissue (Borgå et al., 2005). PCBs are metabolised via the MFO system, specifically the CYP1A and CYP2B subfamilies, which are sensitive to the level and position of chlorination (Warner et al., 2012). CYP1A metabolises PCB congeners lacking *ortho*-, but containing *para*- and *meta*-chlorines with adjacent, unsubstituted *ortho–meta*

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carbons, while CYP2B metabolises PCB congeners with unsubstituted *meta–para* carbons with chlorines at the *ortho* position (White et al., 1997; Kannan and Petrick, 2009).

PCBs can therefore be characterised into three groups, each inducing a separate MFO:

(1) phenobarbital-type (PB-type) PCBs, which induce P450-CYP2B inducers including 2B1 and 2B2, are generally less toxic and more readily excreted,
(2) 3-methylchloranthene-type PCBs (3-MC-type) are inducers of P450-CYP1A. 3-MC-type PCBs are planar molecules with conformational hindrance at the sites for oxygen. This leads to increased stability and decreased detoxification potential, and
(3) PCBs that induce both PB and 3-MC, known as mixed-type PCBs (McFarland and Clarke, 1989; Warner et al., 2012).

PB- and 3MC-induced enzymatic action can both lead to the formation of toxic intermediates (McFarland and Clarke, 1989). PCBs can thus be classified into five structural groups (Table 1) based on their susceptibility to metabolism by the cytochrome P450 system and the presence or absence of vicinal hydrogen atoms (Boon et al., 1997; Borgå et al., 2005). Congeners with unsubstituted *meta–para* positions which induce CYP 2B and 3A like enzymes are more easily biotransformed than congeners with unsubstituted *meta-ortho* positions which activate the CYP 1A-like enzymes (Borgå et al., 2005). The five structural groups are:

I. The most bio-accumulative PCBs with five to seven chlorine atoms that lack vicinal hydrogen atoms in the *ortho–meta* positions, hindering biotransformation processes through enzymatic activity (low metabolic potential).
II. Congeners with vicinal H-atoms in the *ortho-* and *meta*-positions with two or more *ortho*-chlorinated substituents (low metabolic potential).
III. Congeners with vicinal H-atoms in the *ortho-* and *meta*-positions with one or more *ortho*-chlorinated substituents (CYP 1A-like enzyme induction).
IV. Congeners with vicinal H-atoms in the *meta-* and *para*-positions with two or more *ortho*-chlorinated substituents (CYP 2B- and 3A-like enzyme induction).
V. Congeners with vicinal H-atoms in the *meta-* and *para*-positions with three or more *ortho*-chlorinated substituents (CYP 2B- and 3A-like enzyme induction).

The most toxic PCB isomers, due to their co-planar structure, have toxic effect similar to polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Lemieux et al., 2001), and are known as dioxin-like chemicals (DLCs). As with PCDD/Fs, coplanar PCBs bind to the aryl hydrocarbon receptor (AhR) and elicit AhR-mediated biochemical and toxic responses (Behnisch et al., 2001) In birds, Ah-mediated toxicity is suspected to cause reproductive and embryonic effects (Barron et al., 1995), including effects on the size of eggs (Fernie et al., 2000).

Table 1
Assignment of PCBs to different metabolic groups, inducer types and structure, as well as WHO/IPCS toxic equivalency factors (TEFs) for DL-PCBs (McFarland and Clarke, 1989; Boon et al., 1997; Van den Berg et al., 1998; Borgå et al., 2005; Van den Berg et al., 2006).

IUPAC nr	Congener structure	Inducer type	Group	Structure	TEF mammals	TEF birds[b]
28	2,4,4′-trichlorobiphenyl		III	Mono-*ortho*		
31	2,4′,5-trichlorobiphenyl		IV	Mono-*ortho*		
47	2,2′,4,4′-tetrachlorobiphenyl	PB	II	Di-*ortho*		
52	2,2′,5,5′-tetrachlorobiphenyl	PB	IV	Di-*ortho*		
56	2,3,3′,4′-tetrachlorobiphenyl		IV	Mono-*ortho*		
66	2,3′,4,4′-tetrachlorobiphenyl	PB	III	Mono-*ortho*		
74	2,4,4′,5-tetrachlorobiphenyl		III	Mono-*ortho*		
77[b]	3,3′,4,4′-tetrachlorobiphenyl	3-MC	III	Non-*ortho*	0.0001	0.05
81[b]	3,4,4′,5-tetrachlorobiphenyl	Mixed	III	Non-*ortho*	0.0003	0.1
99	2,2′,4,4′,5-pentachlorobiphenyl	PB	II	Di-*ortho*		
101	2,2′,4,5,5′-pentachlorobiphenyl	PB	IV	Di-*ortho*		
105[b]	2,3,3′,4,4′-pentachlorobiphenyl	Mixed	III	Mono-*ortho*	0.00003	0.0001
110	2,3,3′,4′,6-pentachlorobiphenyl		IV	Di-*ortho*		
114[b]	2,3,4,4′5-pentachlorobiphenyl	Mixed	III	Mono-*ortho*	0.00003	0.001
118[b]	2,3′,4,4′,5-pentachlorobiphenyl	Mixed	III	Mono-*ortho*	0.00003	0.00001
123[b]	2′,3,4,4′,5-pentachlorobiphenyl	Mixed	III	Mono-*ortho*	0.00003	0.00001
126[b]	3,3′,4,4′,5-pentachlorobiphenyl	3-MC	III	Non-*ortho*	0.1	0.1
128	2,2′,3,3′4,4′-hexachlorobiphenyl	Mixed	II	Di-*ortho*		
137	2,2′,3,4,4′,5-hexachlorobiphenyl	PB	II	Di-*ortho*		
138	2,2′3,4,4′,5′-hexachlorobiphenyl	Mixed	II	Di-*ortho*		
141	2,2′,3,4,5,5′-hexachlorobiphenyl		IV	Di-*ortho*		
149	2,2′,3,4′,5′,6-hexachlorobiphenyl		V	Tri-*ortho*		
151	2,2′,3,5,5′6-hexachlorobiphenyl	PB	V	Tri-*ortho*		
153	2,2′4,4′,5,5′-hexachlorobiphenyl	PB	I	Di-*ortho*		
156[b]	2,3,3′,4,4′,5-hexachlorobiphenyl	Mixed	III	Mono-*ortho*	0.00003	0.0001
157[b]	2,3,3′,4,4′,5′-hexachlorobiphenyl	Mixed	III	Mono-*ortho*	0.00003	0.0001
167[b]	2,3′,4,4′,5,5′-hexachlorobiphenyl	Mixed	I	Mono-*ortho*	0.00003	0.00001
169[b]	3,3′,4,4′,5,5′-hexachlorobiphenyl	3-MC	I	Non-*ortho*	0.03	0.001
170[a]	2,2′,3,3′,4,4′,5-heptachlorobiphenyl	Mixed	I	Di-*ortho*	0.0001	
180[a]	2,2′,3,4,4′,5,5′-heptachlorobiphenyl	PB	I	Di-*ortho*	0.00001	
183	2,2′,3,4,4′,5′,6-heptachlorobiphenyl	PB	I	Tri-*ortho*		
187	2,2′,3,4′,5,5′,6-heptachlorobiphenyl		I	Tri-*ortho*		
189[b]	2,3,3′,4,4′,5,5′-heptachlorobiphenyl	Mixed	I	Mono-*ortho*	0.00003	0.00001
194	2,2′,3,3′,4,4′,5,5′-octachlorobiphenyl	PB	I	Di-*ortho*		
196	2,2′,3,3′,4,4′,5,6′-octachlorobiphenyl	PB	I	Tri-*ortho*		
199	2,2′,3,3′,4,5,6,6′-octachlorobiphenyl		V	Tetra-*ortho*		
206	2,2′,3,3′,4,4′,5,5′,6′-nonachlorobiphenyl	PB	I	Tri-*ortho*		
209	2,2′,3,3′,4,4′,5,5′,6,6′-octachlorobiphenyl	PB	I	Tetra-*ortho*		

[a] As listed in the WHO/IUPAC interim toxic equivalency factors.
[b] Dioxin-like PCB congeners.

Very little is known about the current levels of PCBs in biota from Africa, and even less in wild birds. The main aim of this study was to assess the occurrence of PCBs in wild bird eggs of various species from different trophic positions. To facilitate the study of different tropic positions, the feeding guild approach was followed. As described by Root (1967), guilds define a group of species that exploit similar environmental resources, in this case focussing on diet. The congener profiles and possible toxicological impact of PCBs in the eggs were then assessed.

2. Materials and methods

The present study was approved by the North–West University Ethics Committee (NWU-EC) (NWU-00055-07-S3) with the necessary permits obtained from provincial governmental departments (HK/P1/08760/001; CPF6 1340). Between October 2008 and January 2009, 77 wild bird eggs, representing 12 different non-migratory, non-endangered species (Table S1), were collected from four sampling areas within the Gauteng and Free State provinces (Fig. 1).

2.1. Sample collection and treatment

The study area was the Gauteng Province (Pretoria, Kempton Park and Soweto) and Vaal Triangle (Vanderbijlpark, Vaalpark and Sasolburg; Fig. 1), which constitutes the major industrial centre of South Africa. Industries include iron and steel works, electricity generation from coal, petrochemical industries, manufacturing of consumer goods, as well as the historic production of organochlorine pesticides (Osibanjo et al., 2002; Quinn et al., 2009).

Eggs were hand-collected and wrapped in aluminium foil precleaned with acetone and hexane. In the laboratory, egg contents (yolk and albumin), were homogenised with an ultrasonic homogeniser (Misonix sonicator 3000, Farmingdale, NY, USA). Eggs were homogenised individually, except for the small eggs of the Cape Turtle Dove, Cape Sparrow and Southern Masked Weaver that had to be pooled. The frozen samples and blank controls were couriered frozen to the Norwegian School of Veterinary Science (NVH) with the necessary export and import permits.

2.2. Analysis

Sample extraction and gas chromatography (GC) analysis were done in the Laboratory of Environmental Toxicology at the NVH, Norway. The egg homogenates were analysed for 34 PCBs (CB-28, -31, -47, -52, -56, -66, -74, -99, -101, 105*, -110, -114*, -118*, -123*, -128, -137, -138, -141, -149, -151, -153, -156, -157, -167, -170, -180, -183, -187, -189, -194, -196, -199,-206 and -209). The mono-*ortho*, dioxin-like (DL) PCBs are marked with *.

The liquid–liquid extraction and clean-up procedures were based on the method of Brevik, 1978, modified as described by Bouwman et al. (2008), Polder et al. (2008a), and Helgason et al. (2008). In short, internal standards (CB-29, -112 and -207; Cambridge Isotope laboratories Inc., Andover, MA, USA), together with 10 mL distiled water, 15 mL acetone, 20 mL cyclohexane and 2 mL NaCl (2% solution) were added to between 2 and 3 g of accurately weighed homogenised egg.

The lipid extraction was performed by ultrasonic homogenisation (4710 Series; Cole Parmer Instruments, Chicago, IL, USA) for 2 min and centrifugation (10 min at 1 643 g). The supernatant was removed and the lipid extraction was repeated with 15 mL acetone: cyclohexane (1:2). The supernatants of both extractions were combined and the volume was adjusted to 5 mL with cyclohexane in a volumetric flask. A 1 mL aliquot of the extract was used for gravimetric lipid determination. Sample extracts were treated twice with concentrated sulphuric acid (purity 96%: Scanpure, Chemscan AS, Elverum, Norway) to remove lipids. The final extracts were concentrated to 0.5 mL for analysis.

A complete description of the GC analysis is given in Murvoll et al., 2006 and Polder et al. (2008b). Briefly, the separation and detection of the non-dioxin-like-PCBs (NDL-PCBs) was performed by high resolution gas chromatography (GC; Agilent 6890 Series gas chromatography system; Agilent Technologies, PA, USA) equipped with an auto sampler (Agilent 7683 Series; Agilent Technologies) and coupled to two ^{63}Ni electron capture detectors (Agilent 6890 μ-ECD). The separation and detection of the dioxin like PCBs (DL-PCBs) was performed by GC (Agilent 6890) equipped with a SPB-5 column (60 m, 0.25 mm, i.d., 0.25 μm film, Supelco) and connected to a low resolution mass spectrometer (LR-MS, Agilent 5973N) operated in selected ion monitoring mode with negative chemical ionisation. The target ions used were: *m/z* 325.8 for CB-112, -123, -118, -114, -105; *m/z* 359.7 for CB-156, -157, -167 and *m/z* 395.7 for CB-189. Limits of detection (LOD) for individual compounds were defined as three times the noise level. To compensate for positive blank values of CB-209 the detection limit for this compound was increased by the mean of the blank values plus two standard deviations.

2.3. Analytical quality control

The laboratory is accredited by the Norwegian Accreditation for testing biological material of animal origin according the requirements of the NS-EN ISO/IEC 17025 (Test 137). The laboratory's analytical quality was successfully approved through international

Fig. 1. Location of sites where wild bird eggs were collected.

inter-calibration and proficiency tests including CRL EUPT-AO 03 pesticides in chicken eggs (2009).

Each sample series included one chicken egg blank, two recovery samples (homogenised chicken eggs, spiked with recovery standards) and three solvent blanks. The repeatability of the GC was confirmed by running a standard after every ten samples while reproducibility of the method was tested through analyses of the laboratory's in-house reference material (seal blubber).

2.4. Data analysis

Calculations were done based on five- or eight-point linear calibration curves with quantification in the linear range for each component. All concentrations are given as ng g^{-1} wet weight (ww) except for comparisons with other studies where lipid-based values were used as lipid weight (ng g^{-1} lw). To assess the influence of trophic levels, birds were divided into five trophic guilds: piscivores (fish eating birds), scavengers (opportunistic feeders), omnivores, herbivores, and granivores (Table S1).

Concentrations below the LOD were assigned the value of half the LOD. Statistical analysis was done using STATISTICA (version 8) and Canoco for Windows (version 4.5). PCAs were performed to elucidate differences in pollution patterns. Log-ratio transformation was applied to the dataset prior to the PCA analysis as described in Howel (2007). In short, the log-ratio of each proportion (p) was determined by dividing each proportion by the geometric mean across the sample (Howel, 2007; Quinn et al., 2009): $\log (p_{ij}/g(p_j))$ where $g(p_j) = (p_{j1}, p_{j2}, p_{j3} \ldots p_{jd})^{1/d}$.

3. Results and discussion

3.1. PCB congener profiles

PCBs were quantified in all eggs (Tables S2 and S3). Eleven PCB congeners (CB-52, -101, -105, -138, -153, -167, -180, -187, -194, -196, and 206) were present in all samples, 22 congeners (CB-28, -31, -47, -66, -74, -99, -110, -114, -118, -123, -128, -137, -141, -149, -151, -156, -157, -170, -183, -189, and -199 and -209) in greater than 70–80% of samples, while, CB-56 was quantified in only 19%.

The dominant congeners in all species were CB-138 (group II), -153 (group III), and -180 (group III), together contributing 25–75% of $_{34}$PCB. This pattern has been seen in many biological matrices including bird tissues worldwide (Boumphrey et al., 1996; Mora, 1996; Humphrey et al., 2000; Dip et al., 2003). The relatively higher levels of these congeners are usually attributed to their recalcitrant and bioaccumulative nature in the environment, as well as their low metabolic degradation potential (McFarland and Clarke, 1989; Borgå et al., 2005; Antoniadou et al., 2007; Luo et al., 2009).

Median PCBs values ranged between 0.92 and 300 ng g^{-1} ww. The White-breasted Cormorant had the highest concentration (415 ng g^{-1} ww), followed by the African Darter (250 ng g^{-1} ww). A higher concentration in piscivores was expected due to bioaccumulation in higher trophic levels (Zimmerman et al., 1997).

Although the industrial uses of PCBs have been phased out since the 1970s, PCBs remain ubiquitous in almost all environmental compartments. In general, it is assumed that levels that are skewed to higher chlorinated PCB congeners are linked to historic applications, whereas the presence of lower chlorinated PCB congeners are more likely from recent or current releases (Breivik et al., 2002). Therefore, the presence of measurable levels of almost all PCB-congeners indicates the presence of current sources in the areas that were sampled.

Because of their high prevalence and persistence, PCB-138, -153 and -180 are ideal PCB congeners for monitoring purposes in wild life from South Africa.

3.2. PCA analysis to investigate the distribution of PCB congeners

In the PCA-biplot (Fig. 2A; factor scores and loadings given in supplementary data Table S4), Factor 1 explained 32% of the variance and was mainly a contrast between the higher chlorinated (CB-138, -153, -156, -157, -170, -180, and -183), with negative scores), and the lower chlorinated PCBs (CB-47, -52, -56, -101, and -110) with positive scores. Piscivores were associated with higher chlorinated PCBs, while insectivores/omnivores and granivores were associated with lower chlorinated PCBs. Birds on a higher trophic level would be expected to have higher chlorinated PCBs (Ólafsdóttir et al., 2001) since lower chlorinated PCBs are more readily metabolised and higher PCBs are more likely to be transferred from prey to predators (Antoniadou et al., 2007). Lower chlorinated PCBs are predominant in non-point source areas through deposition on vegetation and soil (Konstantinou et al., 2000), where granivores, and insectivores are exposed to these congeners.

For further data analysis, PCBs were divided into eight groups according to level of chlorination (Table 1; tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-, and deca- PCB). There was a statistically significant difference between species, feeding guilds, and habitats (one-way ANOVA; $p < 0.05$; Fig. 2B). As with previous studies concerning wild bird eggs, hexa-PCB (28–71%) and hepta-PCB (9–32%) were predominant, likely due to their high persistence (Luo et al., 2009). The order of PCB abundance that followed hexa-CB differed between species. The resistance of PCBs to degradation increases with higher chlorination. However, the extent to which PCBs are metabolised seems to be species-specific (Henry and De Vito, 2003).

There was an increase in PCB concentrations with higher trophic level. Piscivores had the highest levels, followed by scavengers and insectivores/omnivores (Fig. 2B). Due to the physiochemical nature of PCBs, birds will accumulate PCBs from their environment that in turn leads to biomagnification with higher concentrations in higher trophic levels (Warner et al., 2005).

Factor 2 (Fig. 2A) explained 22% of the variance in the data and was a contrast between the less metabolically active group I PCBs with negative scores (PCB-189, -194, -196, -199, -206, and -209) against the more metabolically active group III PCBs which are more readily degraded through CYP 1A enzyme linked reactions (PCB-28, -31, -66, -74, -99, -105, and -123) with positive scores (Borgå et al., 2005). The Southern Masked Weavers and Red-knobbed Coots with positive loadings were separated from the Ardea *spp.* (Black-headed and Grey Heron) with negative loadings on the second axes of the PCA-biplot. This indicates that the Ardea *spp.* eggs contained more persistent PCB congeners when compared to Southern Masked Weaver and Red-knobbed Coot.

3.3. Metabolic and MFO-induction

Metabolic groups as well as PB-type, mixed type inducers (Table 1) and DL-PCBs differed significantly between species, guild and feeding habitat types (one-way ANOVA log transformed data, $p < 0.05$). Group I PCBs were predominant in all eggs with the exception of the Red-knobbed Coot where Group III PCBs were dominant (Fig. 3). In most species, the concentrations of Group V and IV in eggs were negligible compared with Groups I, II and III (Fig. 3). In addition, levels of group V and IV PCBs did not differ significantly between species (one-way ANOVA, $p > 0.05$). The significant difference in the concentration of the various metabolic groups was likely due to differences in trophic level, diet, as well as species specific responses to the individual congeners (Antoniadou et al., 2007).

PB-type, PCBs that induce P450-CYP2B and are generally less toxic and more readily excreted, were predominant in all species

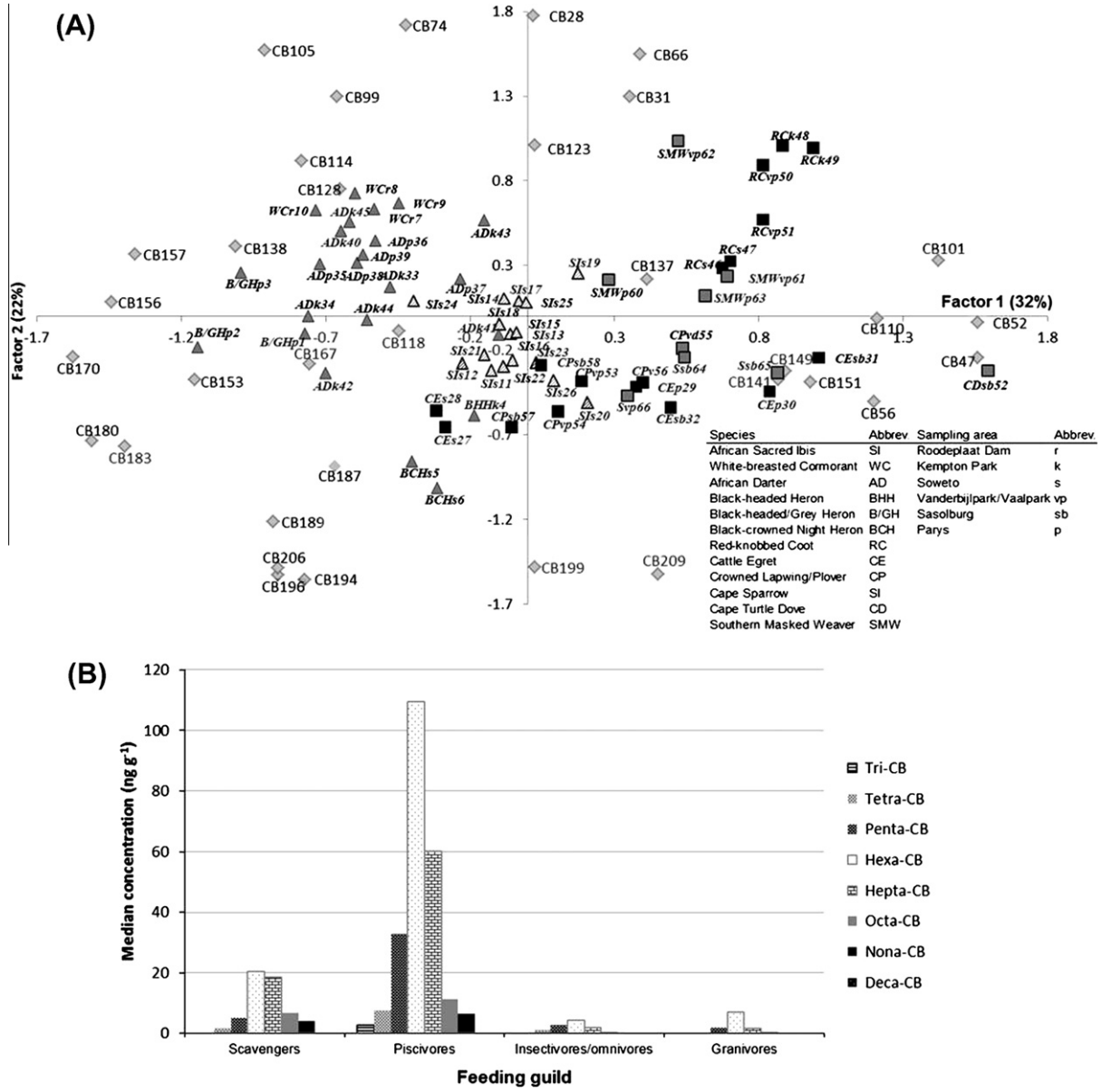

Fig. 2. (A) Biplot of the principle component analysis for PCBs for all wild bird species. The African Sacred Ibis, the only scavenging species, is represented by the triangles (line-pattern), piscivores with triangles (solid grey), insectivores/omnivores with squares (black) and granivores are represented by the squares (grey). (B) The concentration of tri- to deca-CB between the various feeding guilds.

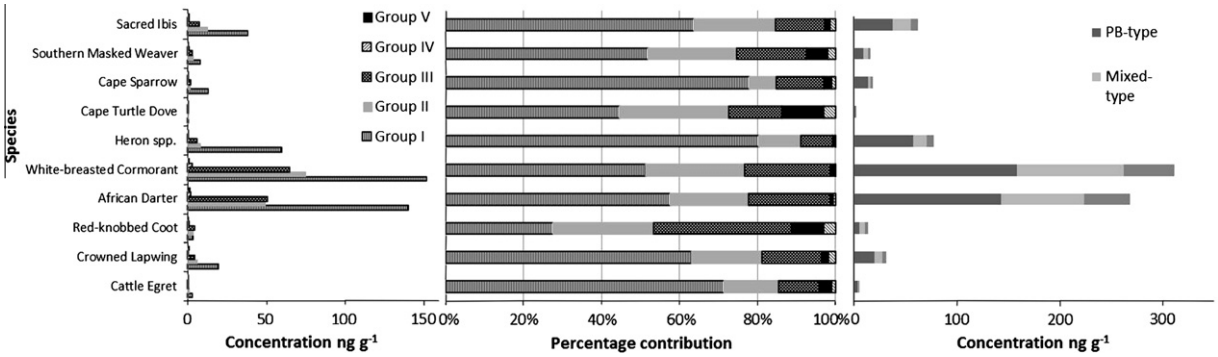

Fig. 3. The concentration and percentage contribution of PCB metabolic groups and PCB groups based on enzyme induction, in wild bird species from South Africa.

(Fig. 3). PB inducible enzymes catalyse the biotransformation of PCBs by inserting oxygen into the molecule (McFarland and Clarke, 1989) and are classified as less toxic. In general, there were higher levels of mixed-type inducers as well as DL-PCBs in piscivores and

Table 2
Mean/median concentrations of PCBs (ng g^{-1}) in bird eggs from South Africa, compared to levels of PCBs measured in eggs and fat tissue in other published studies.

Common name	Scientific name	Feeding guild	Matrix	Years of sampling	*n*	∑PCBs	No. of congeners	Study area	Source
European Shag	*Phalacrocorax aristotelis*	Piscivore	Yolk sac	2002	30	16128	23	Norway	Murvoll et al., 2006
Great Tit	*Parus major*	Insectivore	Eggs	2000	38	4778	21	Antwerp, Belgium	Van den Steen et al., 2006
Blue Heron	*Ardea herodias*	Piscivore	Eggs	2000	10	19 000	31–51	Canada	Harris et al., 2003
Blue Heron	*Ardea herodias*	Piscivore	Eggs	2000	10	8000	31–51	Canada	Harris et al., 2003
Little Egret	*Egretta gazella*	Insectivore	Eggs	2000	20	14769	NA	Hong Kong	Conell et al., 2003
Black-crowned Night Heron	*Nycticorax nycticorax*	Piscivore	Eggs	2000	20	3433	NA	Hong Kong	Conell et al., 2003
Herring Cull	*Larus argentatus*	Scavenger	Eggs	2003	5	11 596	20	Northern Norway	Helgason et al., 2008
Black-legged Kittiwake	*Rissa tridactyla*	Piscivore	Eggs	2003	5	7254	20	Northern Norway	Helgason et al., 2008
Common Guillemot	*Uria aalge*	Piscivore	Eggs	2003	5	2333	20	Northern Norway	Helgason et al., 2008
Atlantic Puffin	*Fratercula arctica*	Piscivore	Eggs	2003	5	4594	20	Northern Norway	Helgason et al., 2008
Northern Lapwing	*Vanellus vanellus*	Insectivore	Eggs	2006	14	4360	20	Antwerp, Belgium	Dauwe et al., 2009
Great Tit	*Parus major*	Insectivore	Eggs	2006	14	2760	20	Antwerp, Belgium	Dauwe et al., 2009
Mediterranean Gull	*Larus melanocephalus*	Scavenger	Eggs	2006	6	1770	20	Antwerp, Belgium	Dauwe et al., 2009
Black-necked Grebe	*Podiceps nigricollis*	Piscivore	Fat	1992–1994	1	390	7	Nakdong River, Korea	Choi et al., 2001
Great Knot	*Calidris tenuirostris*	Invertebrate	Fat	1992–1995	1	159	7	Nakdong River, Korea	Choi et al., 2001
Sanderling	*Crocethia alba*	Invertebrate	Fat	1992–1996	5	3508	7	Nakdong River, Korea	Choi et al., 2001
Greenshank	*Tringa nebularia*	Invertebrate	Fat	1992–1997	3	970	7	Nakdong River, Korea	Choi et al., 2001
Bar-tailed Godwit	*Limosa lapponica*	Piscivore	Fat	1992–1998	5	1596	7	Nakdong River, Korea	Choi et al., 2001
Black-headed Gull	*Larus ridibundus*	Scavenger	Fat	1992–1999	7	6005	7	Nakdong River, Korea	Choi et al., 2001
Herring Gull	*Larus argentatus*	Scavenger	Fat	1992–2000	4	7818	7	Nakdong River, Korea	Choi et al., 2001
Common Gull	*Larus canus*	Scavenger	Fat	1992–2001	10	3744	7	Nakdong River, Korea	Choi et al., 2001
Common Tern	*Sterna hirundo*	Piscivore	Fat	1992–2002	2	1326	7	Nakdong River, Korea	Choi et al., 2001
Little Tern	*Sterna albifrons*	Piscivore	Fat	1992–2003	3	2621	7	Nakdong River, Korea	Choi et al., 2001
Kentish Plover	*Charadrius alexandrinus*	Insectivore	Eggs	2008	8	181	68	China	Gao et al., 2009
Common Coot	*Fulica atra*	Omnivore	Eggs	2008	4	26	68	China	Gao et al., 2009
Common Tern	*Sterna hirundo*	Piscivore	Eggs	2008	9	216	68	China	Gao et al., 2009
African Darter	*Anhinga rufa*	Piscivore	Eggs	2004–2005	14	5070	34	South Africa	Bouwman et al., 2008
Reed Cormorant	*Phalacrocorax africanus*	Piscivore	Eggs	2004–2005	3	2657	34	South Africa	Bouwman et al., 2008
Cattle Egret	*Bubulcus ibis*	Insectivore	Eggs	2004–2005	20	89	34	South Africa	Bouwman et al., 2008
African Sacred Ibis	*Threskiornis arthiopicus*	Scavenger	Eggs	2004–2005	1	1048	34	South Africa	Bouwman et al., 2008
Crowned Plover	*Vanellus cornonatus*	Insectivore	Eggs	2004–2005	1	90	34	South Africa	Bouwman et al., 2008
Little Grebe	*Tachybaptus ruficollis*	Piscivore	Eggs	2004–2005	1	197	34	South Africa	Bouwman et al., 2008
White-fronted Plover	*Charadrius marginatus*	Insectivore	Eggs	2004–2005	1	302	34	South Africa	Bouwman et al., 2008
Kelp Gull	*Larus dominicanus*	Scavenger	Eggs	2004–2005	1	1029	34	South Africa	Bouwman et al., 2008
African Sacred Ibis	*Threskiornis aethiopicus*	Scavenger	Eggs	2008–2009	16	1088	34	South Africa	Present study
African Darter	*Anhinga rufa*	Piscivore	Eggs	2008–2009	13	4601	34	South Africa	Present study
White-breasted Cormorant	*Phalacrocorax carbo*	Piscivore	Eggs	2008–2009	4	7740	34	South Africa	Present study
Heron *sp.*	_	Piscivore	Eggs	2008–2009	6	1112	34	South Africa	Present study
Cattle Egret	*Bubulcus Ibis*	Insectivore	Eggs	2008–2009	6	63	34	South Africa	Present study
Crowned Lapwing	*Vanellus coronatus*	Insectivore	Eggs	2008–2009	6	278	34	South Africa	Present study
Red-knobbed Coot	*Fulica cristata*	Omnivore	Eggs	2008–2009	6	100	34	South Africa	Present study
Cape Sparrow	*Passer melanurus*	Granivore	Eggs	2008–2009	9	248	34	South Africa	Present study
Southern Masked Weaver	*Ploceus velatus*	Granivore	Eggs	2008–2009	8	349	34	South Africa	Present study

scavengers, indicating increased potential risk to these bird species (Fig. 3).

3.4. Comparisons

In general, PCB concentrations in wild bird eggs studied were lower than that in reviewed literature (Table 2). Concentrations here were comparable with those reported by Bouwman et al. (2008). Concentrations in eggs of piscivore species were approximately half of those measured in the European Shag from Norway (Murvoll et al., 2006) and Blue Herons from an industrialised area in Canada (Harris et al., 2003). However, they were three times higher than levels measured in piscivore eggs from Korea (Choi et al., 2001). All other piscivores eggs in Table 2 were in the same

range or order of magnitude as the present study. Levels in insectivores were in the same range as those measured in the Kentish Plover from China (Gao et al., 2009), but between 3% and 53% lower than all other literature reviewed. For the scavengers, levels were in the same range as the Mediterranean Gull from Belgium (Dauwe et al., 2009), but 3–10% less than in other studies.

3.5. Toxicity assessment

Although PCBs were one of the first chemical classes directly linked to decreases in wildlife populations (Jensen, 1966), there is still a lack of data on the relative importance of non-dioxin like PCBs in wild bird populations (Henry and De Vito, 2003). Reproductive failures have been linked to PCBs concentration of 1.6–10 $\mu g\, g^{-1}$ ww in cormorant and heron species (Antoniadou et al., 2007). Decreased hatchling success has been reported at concentrations of 1–25 $\mu g\, g^{-1}$ ww for chickens, cormorants, and eagles (Brunström and Halldin, 2000). Mortality in birds has been associated with concentrations between 90 and 470 $\mu g\, g^{-1}$ ww (Guruge et al., 1997).

The "no observed effects level" (NOEL) of 4 $\mu g\, g^{-1}$ ww in eggs as proposed by Brunström and Halldin (2000), was used to assess the risk of PCBs in the current study. In the present study, the highest $_{34}$PCB concentration was in a White-Breasted Cormorant egg (290 $ng\, g^{-1}$, Table S7), 13 times lower than the NOEL, indicating low risk of reproductive failure or mortality from the current level of PCBs. Birds with increased levels of PCBs have been linked with behavioural (hatching and rearing) impairment (Bustnes et al., 2001), altered hormone function and increased stress responses (Fry, 1995; Dawson, 2000). These effects all indicate possible negative health effects on wild birds exposed to PCBs. However since these effects are at a sub-lethal level they cannot easily be quantified, an aspect that needs urgent research in African conditions.

4. Conclusions and recommendations

All species had quantifiable levels of PCBs, with 88% of the congeners measured being found in more than 80% of samples. PCB levels were highest in piscivore species that also had the highest percentage of DL-PCBs. Metabolic group I PCBs were more prevalent, likely due to resistance to degradation, whereas the more biodegradable group IV and V PCBs were present in low levels. The PB-type inducer PCBs were more prevalent than mixed-type PCBs, indicating a prevalence of less toxic PCBs in wild bird eggs. Higher chlorinated congeners were more prevalent in higher trophic levels while lower chlorinated congeners were associated with lower trophic levels.

The high prevalence of CB-138, -153 and -180 found in this study make them ideal candidates for monitoring PCBs in the South African environment specifically. The difference between the higher and lower chlorinated PCBs according to trophic position, requires further research. The presence of mono-*ortho* PCBs at appreciable levels motivates for the continued assessment of dioxin-like chemicals in wild bird eggs.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.09.016.

References

Antoniadou, V., Konstantinou, I.K., Sakellarides, T.M., Albanis, T.A., Bintoudi, E., 2007. PCB levels and accumulation patterns in waterbird eggs and in their prey at Lake Kerkinin, a North-eastern Mediterranean wetland of international importance. Arch. Environ. Contam. Toxicol. 53, 249–260.

Barron, M.G., Galbraith, H., Beltman, D., 1995. Comparative reproductive and developmental toxicology of PCBs in birds. Comp. Biochem. Physiol. C 112 (1), 1–14.

Behnisch, P.A., Hosoe, K., Shiozaki, K., Ozaki, H., Nakamura, K., Sakai, S.I., 2001. Bioanalytical screening methods for dioxins and dioxin-like compounds – a review of bioassay/biomarker technology. Environ. Int. 27, 413–439.

Boon, J.P., van der Meer, J., Allchin, C.R., Law, R.J., Klungsøyr, J., Leonards, P.E.G., Spliid, H., Storr-Hansen, E., Mckenzie, C., Wells, D.E., 1997. Concentration-dependant changes of PCB patterns in fish-eating mammals: structural evidence for induction of cytochrome P450. Arch. Environ. Contam. Toxicol. 33 (3), 298–311.

Borgå, K., Wolkers, H., Skaare, J.U., Hop, H., Muir, D.C.G., Gabrielsen, G.W., 2005. Bioaccumulation of PCBs in Arctic seabirds: influence of dietary exposure and congener biotransformation. Environ. Pollut. 134, 397–409.

Boumphrey, R.S., Harrad, S.J., Jones, K.C., Osborn, D., 1996. Polychlorinated biphenyl congener patterns in tissue from a selection of British birds. Arch. Environ. Contam. Toxicol. 25, 346–352.

Bouwman, H., Polder, A., Venter, B., Skaare, J.U., 2008. Organochlorine contaminants in cormorant, darter, egret and ibis eggs from South Africa. Chemosphere 71, 227–241.

Brevik, E.M., 1978. Gas chromatographic method for the determination of organochlorine pesticides in human milk. Bull. Environ. Contam. Toxicol. 19, 281–286.

Breivik, K., Sweetman, A., Pacyna, J.M., Jones, K.C., 2002. Towards a global historical emission inventory for selected PCB congeners-a mass balance approach 2. Emissions, Sci. Total. Environ. 290, 199–224.

Bustnes, J.O., Bakken, V., Erickstad, K.E., Mehlum, F., Skaare, J.U., 2001. Patterns of incubation and nest-site attentiveness in relation to organochlorine (PCB) contamination in glaucous gulls. J. Appl. Ecol. 38, 791–801.

Brunström, B., Halldin, K., 2000. Ecotoxicological risk assessment of environmental pollutants in the Arctic. Toxicol. Lett. 112, 111–118.

Choi, J.W., Matsunds, M., Kawano, M., Min, B.Y., Wakimoto, T., 2001. Accumulation profiles of persistent organochlorines in waterbirds from an estuary in Korea. Arch. Environ. Contam. Toxicol. 41, 353–366.

Conell, D.W., Fung, C.N., Minh, T.B., Tanabe, S., Lam, P.K.S., Wong, B.S.F., Lam, M.H.W., Wong, L.C., Wu, R.S.S., Richardson, B.J., 2003. Risk to breeding success of fish-eating Ardeids due to persistent organic contaminants in Hong Kong: evidence from organochlorine compounds in eggs. Water Res. 37, 459–467.

Dawson, A., 2000. Mechanism of endocrine disruption with particular reference to occurrence in avian wildlife: a review. Ecotoxicology 9, 59–69.

Dauwe, T., Van den Steen, E., Jaspers, V.L.B., Maes, K., Covaci, A., Eens, M., 2009. Interspecific differences in concentrations and congener profiles of chlorinated and brominated organic pollutants in three insectivorous bird species. Environ. Int. 35, 369–375.

Denison, M.S., Whitlock Jr., J.P., 1995. Xenobiotic-inducible transcription of cytochrome P450 genes. J. Biol. Chem. 270 (31), 18175–18178.

Dip, R., Hegglin, D., Deplazes, P., Dafflon, O., Koch, H., Naegeli, H., 2003. Age-and sex-dependent distribution of persistent organochlorine pollutants in urban foxes. Environ. Health. Persp. 111 (13), 1608–1612.

Fernie, K.J., Bortolotti, G.R., Smits, J.E., Wilson, J., Drouillard, K.G., Bird, D.M., 2000. Changes in egg composition of American Kestrels exposed to dietary polychlorinated biphenyls. J. Toxicol. Environ. Health, Part A 60, 291–303.

Fry, D.M., 1995. Reproductive effects in birds exposed to pesticides and industrial chemicals. Environ. Health Persp. 103, 165–171.

Gao, F., Luo, X.-J., Yang, Z.-F., Wang, X.-M., Mai, B.-X., 2009. Brominated flame retardants, polychlorinated biphenyls, and organochlorine pesticides in bird eggs from the Yellow River Delta. North-China. Environ. Sci. Technol. 43, 6956–6962.

Guruge, K.S., Tanabe, S., Fukuda, M., Yamagishi, S., Tatsukawas, R., 1997. Accumulation pattern of persistent organochlorine residues in Common Cormorants (*Phalacrocorax carbo*) from Japan. Mar. Pollut. Bull. 34 (3), 186–193.

Harris, M.L., Elliot, J.E., Butler, R.W., Wilson, L.K., 2003. Reproductive success and chlorinated hydrocarbons contamination of resident great blue herons (*Ardea herodias*) from coastal British Columbia, Canada, 1977–2000. Environ. Pollut. 121, 207–227.

Helgason, L.B., Barett, R., Lie, E., Polder, A., Skaare, J.U., Gabrielsen, G.W., 2008. Levels and temporal trends (1983–2003) of persistent organic pollutants (POPs) and

mercury (Hg) in seabird eggs from Northern Norway. Environ. Pollut. 155, 190–198.
Henry, T.R., De Vito, M.J., 2003. Non-dioxin-like PCBs: effects and consideration in ecological risk assessments. In: Report NCEA-C-1340, ERASC-003, United States Environmental Protection Agency, Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory Office of Research and Development. Cincinnati, Ohio.
Howel, D., 2007. Multivariate data analysis of pollutant profiles: PCB levels across Europe. Chemosphere 67, 1300–1307.
Humphrey, H.E.B., Gardiner, J.C., Pandya, J.R., Sweeney, A.M., Gasior, D.M., McCaffrey, R.J., Schantz, S.L., 2000. PCB congener profile in the serum of humans consuming Great Lakes Fish. Environ. Health Persp. 108 (2), 167–172.
Jensen, S., 1966. Report of a new chemical hazard. New Sci. 32, 612.
Kannan, N., Petrick, 2009. Ecological risk assessment using high resolution analysis of polychlorinated biphenyls (PCBs). In: Interdisciplinary studies on environmental chemistry – environmental research in Asia. Terrapub. pp. 99–109.
Konstantinou, I.K., Gouther, V., Albanis, T.A., 2000. The incidence of polychlorinated biphenyl and organochlorine pesticide residues in the eggs of the cormorant (*Phalacrocorax carbo* sinensis): an evaluation of the situation in four Greek wetlands of international importance. Sci. Total. Env. 257, 61–79.
Lemieux, P.M., Lee, C.W., Ryan, J.V., Lutes, C.C., 2001. Bench-scale studies on the simultaneous formation of PCBs and PCDD/Fs from combustion systems. Waste Manage. 21, 419–425.
Luo, S.-J., Zhang, X.-L., Liu, J., Wu, J.-P., Luo, Y., Chen, S.J., Mai, B.-X., Yang, Z.-Y., 2009. Persistent halogenated compounds in waterbirds from an e-waste recycling region in South China. Environ. Sci. Technol. 43 (2), 306–311.
McFarland, V.A., Clarke, J.U., 1989. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. Environ. Health Persp. 81, 225–239.
Mora, M.A., 1996. Congener-specific polychlorinated biphenyl patterns in eggs of aquatic birds from the lower Laguna Madre. Texas. Environ. Toxicol. Chem. 15 (6), 1003–1010.
Murvoll, K.M., Skaare, J.U., Anderssen, E., Jenssen, B.M., 2006. Exposure and effects of persistent organic pollutants in European shag (*Phalacrocorax aristotelis*) hatchlings from the coast. Environ. Toxicol. Chem. 25 (1), 190–198.
Ólafsdóttir, K., Peterson, A.E., Magnúsdóttir, E.V., Björnsson, T., Jóhannesson, T., 2001. Persistent organochlorine levels in six prey species of the gyrfalcon Falco rusticolus in Iceland. Environ. Pollut. 112, 245–251.
Osibanjo, O., Bouwman, H., Bashir, N.H.H., Okond'Ahoka, J., Choong Kwet Yve, R., Onyoyo, H.A., 2002. Regionally based assessment of persistent toxic substances. In: Sub-Saharan Africa regional report. UNEP Chemicals, Geneva.
Polder, A., Venter, B., Skaare, J.U., Bouwman, H., 2008a. Polybrominated diphenyl ethers and HBCD in bird eggs of South Africa. Chemosphere 73, 148–154.
Polder, A., Gabrielsen, G.W., Odland, J.Ø., Tkachev, A., Savinova, T.N., Løken, K.B., Skaare, J.U., 2008b. Spatial and temporal changes of chlorinated pesticides, PCBs, dioxins (PCDDs/PCDFs) and brominated flame retardants in human breast milk from Northern Russia. Sci. Total. Environ. 391, 41–54.
Quinn, L., Pieters, R., Nieuwoudt, C., Borgen, A.R., Kylin, H., Bouwman, H., 2009. Distribution profiles of selected organic pollutants in soils and sediments of industrial, residential and agricultural areas of South Africa. J. Environ. Monitor. 11, 1647–1657.
Root, R.B., 1967. The nich exploitation pattern of the blue–gray gnatcatcher. Ecol. Monogr. 37 (4), 317–350.
Takasuga, T., Scnthilkumar, K., Matsumura, T., Shiozaki, K., Sakai, S., 2006. Isotope dilution analysis of polychlorinated biphenyls (PCBs) in transformer oil and global commercial PCB formulations by high resolution mass spectrometry. Chemosphere 62, 469–484.
UNEP Chemicals., 1999. Guidelines for the identification of PCBs and materials containing PCBs. <www.chem.unep.ch/publications/pdf/GuiddPCB.pdf04/10/201>.
Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farkland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Toyama, C., Tristher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World Health Organisation re-evaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93 (2), 223–241.
Van den Berg, M., Birnbaum, L., Bosveld, A.T., Brunstrom, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, SW., Kybiak, T., Larsen, J.C., Van Leeuwen, R.F.X., Liem, A.K., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs and PCDFs for humans and wildlife. Environ. Health. Persp. 106, 775–792.
Van den Steen, E., Dauwe, T., Jaspers, V.L.B., Pinxten, R., Eens, M., 2006. Within- and among-clutch variation of organohalogenated contaminants in eggs of great tits (Parus major). Environ. Pollut. 144, 355–359.
Warner, J., Osuch, J.R., Karmaus, W., Landgraf, J.R., Taffe, B., O'Keefe, M., Mikucki, D., Haan, P., 2012. Common classification schemes for PCB congeners and the gene expression of CYP17, CYP 19, ESR1 and ESR2. Sci. Total. Environ. 414, 81–89.
Warner, N.A., Norstrom, R.J., Wong, C.S., Fisks, A.T., 2005. Enantiomeric fractions of chiral polychlorinated biphenyls provide insights on biotransformation capacity of arctic biota. Environ. Toxicol. Chem. 24 (11), 2763–2767.
White, R.D., Shea, D., Stegeman, J.J., 1997. Metabolism of the aryl hydrocarbon receptor agonist 3,3′,4,4′-tetrachlorobiphenyl by marine fish scup (*Stenotomus chrysops*) *in vivo* and *in vitro*. Drug. Metab. Dispos. 25 (5), 564–572.
Zimmerman, G., Dietrich, D.R., Schmid, P., Schlatter, C., 1997. Congener-specific bioaccumulation of PCBs in different water bird species. Chemosphere 43, 1379–1388.