Assessment of organic pollutants in selected wild and domesticated bird eggs from Gauteng, South Africa

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Acknowledgements

Science is facts, just as houses are made of stone, so science is made of facts, but a pile of stones is not a house and a collection of facts is not necessarily science. Jules Henri Poincaré (1854-1912).

To build a house out of a pile of stones one needs labour, resources, and expertise. The expertise and guidance of others is ultimately the foundation on which the remainder of the structure rests. The completion of this thesis would not have been possible without a score of people's assistance, nor without institutions that provided facilities, resources and financial support.

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Every good and perfect gift is from above, coming down from the Father of the heavenly lights, who does not change like shifting shadows. James 1:17 (New International Version)

I would like to dedicate this thesis in loving memory of Thomas Charles William Rogers.
The greatest gift that one can receive is the quest and love for knowledge, knowledge with truth and meaning.

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Abstract

Polybrominated flame retardants (BFRs), organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) were analysed in eggs of various wild bird species from industrialised areas in South Africa. Eggs were collected during the 2008 – 2009 breeding season, homogenised and sent to the Norwegian School of Veterinary Science (NVH) for gas chromatography-mass spectrometry (GC-MS) analysis. The concentration, contamination profile, and risk assessment were conducted for each pollutant class, while effects of species-specific variation, feeding guild, and feeding habitat were investigated.

Levels of BFRs ranged between 2.6 – 44 ng g^{-1} wet mass (wm). The predominant congeners were BDE-153, -154, - 183 and -47. Results indicated species, in close contact to humans, had higher levels of BFRs, even at lower trophic levels. Therefore, diet was not the primary route of exposure. High concentrations and the occurrence of nona-PBDE congeners and HBCD indicated exposure to current use BFRs. There were measurable levels of OCPs and PCBs in all eggs analysed. Median OCP concentration ranged from 4.2 – 623 ng g^{-1} wm. DDE was the predominant compound in all species with the exception of the Crowned Lapwing (*Vanellus coronatus)* where chlordanes were predominant. This may indicate a species-specific attribute in the metabolic efficiency or diet of the genus, since these findings have been reported elsewhere in literature. Congener profiles indicated historic sources of lindane and DDT, while low levels of *p,p'*-DDT in al species indicate long-range or atmospheric transport. Even though levels of *p,p'*-DDE were approaching toxicological thresholds, no eggshell thinning was evident. Concentrations of OCPs and PCBs showed an increase with increasing tophic level. PCB concentrations ranged between 0.9 – 296.4 ng g^{-1} wm. When studying the metabolic potential of PCBs, 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 were not analysed. These congeners aslo could impact on the toxic potential of PCBs in wild bird eggs.

Principle component analysis (PCA) indicated that variances within datasets could be attributed to congener profiles within species as they were affected by exposure, diet, position in the food web, and association with human activities. Although the individual groups of organohalogens were below no observed effect levels (NOELs), negative effects could occur

through interactions of various compounds with each other, as well as the unique exposure profiles of South African bird populations.

To assess the dietary exposure of low-income human populations living close to large industries, the occurrence of organohalogens was investigated in backyard chicken eggs. Levels of dioxins in these eggs were above the European Union (EU) recommended limits, whereas BFRs and OCPs levels were below levels of concern. Nevertheless, areas where DDT is actively applied to dwellings for malaria control should be urgently investigated.

The presence of measureable levels of all the compounds considered, indicate an environment seriously impacted by anthropogenic activity that in the long term could negatively affect both the environment and human health, if it has not already done so.

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Keywords: PBDEs; HBCD; DDT; DDE; lindane; HCH; HCB; chlordane; oxychlordane; mirex; PCB; PCDD; PCDF; chicken eggs; wild bird eggs; habitat; trophic level; feeding guild; PCA; risk assessment.

Contents

Abbreviations

3-MC-type 3-Methylchloranthene-type PCB

A

AD African Darter

ADI Average daily intake

ANOVA Analysis of variance

ATSDR Agency for Toxic Substances and Disease Registry

B

BCH Black-crowned Night Heron

BFRs Brominated flame retardants

B/GH Black-headed/Grey Heron

BHH Black-headed Heron

C

CITES Convention on International Trade in Endangered Species

CD Cape Turtle Dove

CDC Centre for Disease Control

CE Cattle Egret

CP Crowned Lapwing/Plover

D

DDA 2,2-bis(p-chlorophenyl)acetic acid

DDD 1,1-dichloro-2,2(p-chlorophenyl)ethane

DDE 1,1-dichloro-2,2(p-chlorophenyl)ethylene

DDT 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane

DLC Dioxin-like chemicals

DL-PCB Dioxin-like PCBs

dm Dry mass

E

ECD Electron capture detector

EDC Endocrine disrupting chemical

EPA Environmental Protection Agency

EU European Union

F

fm Fresh mass

G

GABA Gamma-amino butyric acid

GC Gas chromatography

H

H Hydrogen

HBCD hexabromocyclododecane

HCB hexachlorobenzene

HCH Hexachlorocyclohexane

Hepta-BDE Heptabromodiphenyl ether

Hexa-BDE Hexabromodiphenyl ether

HPDE High density polyethylene

HR High resolution

I

IPCS International Program on Chemical Safety

IRS Indoor residual spraying

IRIS Integrated Risk Information System

IUCN International Union for Conservation of Nature

K

k Kempton Park

L

LOD Limit of detection

LOQ Limit of quantification

LR Low resolution

M

MFO Mixed function oxidase

MS Mass spectrometry

N

NCI Negative chemical ionisation
ND No detects
NOEL No observed effect level
NRF National Research Foundation
NVH Norwegian School of veterinary Science

O

OC Organochlorine
OCP Organochlorine pesticides

P

p Parys
PAH Polyaromatic hydrocarbons
PB-type Phenobarbital type
PBDE Polybrominated diphenyl ethers
PCA Principle component analysis
PCB Polychlorinated biphenyl
PCDD Polychlorinated dibenzo-p-dioxin
PCDF Polychlorinated dibenzofurans
PE Polypropylene
PeCB Pentachlorobenzene
Penta-BDE Pentabromodiphenyl ether
PFOS Perfluorooctane sulfonic acid
PFOSF Perfluorooctane sulfanyl fluoride
POPs Persistent organic pollutants
PTV Programable temperature vaporization
PVC Polychlorine vinyl

R

R Roodeplaat Dam
RC Red-knobbed Coot
RCN Research Council of Norway
REACH Registration, Evaluation and Authorisation of Chemicals

S

s Soweto
S Cape Sparrow
sb Sasolburg
SC Stockholm Convention
SI African Sacred Ibis
SIM Seleted ion monitoring
SMW Southern Masked Weaver

T

TBBPA Tetrabromobisphenol A
TCDD 2,3,7,8-tetrachloro-dibenzo-p-dioxin
TDI Tolerable daily intake
TEF Toxic equivalency factor
TEQ Toxic equivalent
Tetra-BDE Tetrabromodiphenyl ether

U

UNEP United Nations Environmental Program
USA United Stated of America

V

vp Vanderbijlpark/Vaal Park

W

WC White-breasted Cormorant
WHO World Health Organisation
wm Wet mass

X

XPLE Cross-linked polyethylene

1 Introduction

Persistent organic pollutants (POPs) are toxic and organic compounds that undergo long-range environmental transport and therefore represent a global contamination problem (Fu *et al.,* 2003; Breivik *et al*., 2004; Hirano *et al*., 2007; De Wit *et al*., 2010). In recent years, POPs have come under scrutiny from scientific, governmental, and non-governmental groups (Lerche *et al*., 2002) as one of the most dangerous groups of environmental pollutants (Lebedev *et al.*, 1998). The monitoring and study of POPs and other emerging organic pollutants are of great importance due the adverse effects these chemicals can exhibit on both the environment, and human health. Exposure to POPs can lead to health effects including cancer, birth defects and impaired neurological development (Kohn, 1995; Handberg; 1996; Langer, 1998). These chemicals are bio-accumulative and tend to bio-concentrate in the food web due to a high affinity for lipophilic conditions and resistance to degradation (Van Wyk *et al*., 2001; Van den Steen *et al*., 2006). Some POPs have been produced commercially and others are formed as unintentional by-products of anthropogenic activity.

The inherent global risk posed by POPs culminated in the development of the Stockholm Convention (SC). The main aim of the SC is to protect humans and the environment from chemicals that are persistent, bio-accumulate, and tend to become geographically widely distributed (Stockholm Convention on POPs, 2009). As a party South Africa has the responsibility, according to Article 11, to undertake appropriate research, development, monitoring and cooperation pertaining to POPs. The continued lack of data in this regard is the main motivation for undertaking research into the status of POPs and emerging POPs within the South African context (Bouwman, 2004). Although many of these compounds have been banned or their use seriously restricted, POPs are still present and detected in environmental media.

The extensive use of organochlorine pesticides (OCPs) and industrial applications of compounds such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) resulted in negative effects on terrestrial and aquatic ecosystems (Sakellarides *et al*., 2006). Birds are specifically sensitive to the classical POPs as indicated by the population declines in Europe and North America during the 1950s and 1960s that were linked to the widespread use of OCPs (Prest *et al*., 1970; Nygård, 1999; Dawson, 2000; Carson, 2002; Walker, 2009). It is widely accepted that these declines were due to eggshell thinning, resulting

in increased embryo mortality (Dawson, 2000). Eggshell thinning is still attributed to current exposures of historically used POPs (Bouwman *et al*., 2008). Therefore, birds are good indicators of contamination (Medvedev & Markove, 1995; Lebedev *et al*., 1998; Tanabe & Subramanian, 2003; Jaspers *et al*., 2005) and have been used since the 1970s as monitoring species for organochlorine (OC) contamination (Norstrom *et al*., 1980) in both terrestrial and aquatic ecosystems.

South Africa has a rich biodiversity of avian species, and is currently one of the top birding destinations in the world. Tourism is one of the greatest contributors to the South African economy, with established birding routes generating $ 6.4 million (R 46.8 million) annually (Birdlife International, 2008). Birding routes are but one of the many ways that tourists use to view the rich bird diversity in South Africa. To date, more than 900 different bird species have been recorded in South Africa, representing 10% of the bird species in the world (South African Bird Atlas Project, 2009). Of the bird species occurring in South Africa and Lesotho, 103 species are globally or nationally threatened (Bonn *et al*., 2002). Species occurring in these areas include 59 that are endemic or near-endemic of which 22 are threatened (Bonn *et al*., 2002). This makes conservation of existing species crucial from both an ecologic and an economic viewpoint.

Previously, studies concerning POPs in South Africa identified these chemicals in wild bird populations (Van Wyk *et al*., 2001; Bouwman *et al*., 2008; Polder *et al*., 2008a) at levels warranting further investigation. An additional factor that has to be considered when assessing the risk that POPs have for avian populations, are the unique characteristics in the life histories of South African bird populations. An example of one such characteristic is the lower rate of migration in piscivorous species, such as herons. Palaearctic populations migrate seasonally, whereas southern Africa herons are local residents year-round due to the temperate climate (Hockey *et al*., 2005). Furthermore, bird species from the southern hemisphere, with its predominant temperate to tropical climates, have smaller clutch sizes (Evans *et al*., 2005), slower developmental rates and longer life spans (Ghalambor & Martin, 2001) when compared to birds from the northern hemisphere. These combinations of factors may lead to different exposure assessments between birds in temperate and tropical zones when compared to Europe and areas within North America.

POPs and other contaminants, such as polyaromatic hydrocarbons (PAHs) and heavy metals are also present in domesticated birds such as poultry (Van Overmeire *et al*., 2009). In South Africa, as with many developing countries, poultry is an important food source (Swatson *et al*., 2009). People living in poorer communities with will supplement their diet and their

income with backyard poultry. In general backyard chickens can be confined to coops or free roaming. Additionally, backyard chickens are often fed table scraps as well as bought feed. If said chickens are free roaming these birds will scavenge food such as insects and plant material readily available from their surroundings. Backyard chickens also show increased geophagy compared to commercial chickens (Van Overmeire *et al*., 2006; Covaci *et al*., 2009). For these reason, the human consumption of home produced eggs is often linked with increased dietary intake of POPs, when compared to the consumption of commercial eggs. This could be a serious problem in South Africa where low-income communities are found close to industries and where measurable levels of POPs have been found in the soil where these communities recide (Nieuwoudt *et al*., 2009; Quinn *et al*., 2009).

The aim of this study was to investigate the presence and levels of POPs in wild bird populations as well as in backyard chicken eggs, in the Gauteng region, the most industrialised area in South Africa. From this data, preliminary risk assessments of human dietary intake of backyard chicken eggs as well as a risk assessment for bird populations were undertaken. By doing so this study contributes towards the SC aims for South Africa.

1.1. BIRDS AS INDICATORS OF ENVIRONMENTAL POLLUTANTS

Birds are specifically sensitive to chlorinated compounds (Van Wyk *et al.*, 2001) and are easily spotted. Additionally, predatory birds are high on the trophic level within the food web (Jasper *et al*., 2005) making them good indicator species for POPs. POPs levels measured in birds can also be used in comparisons on regional and global scales (Barber *et al*., 2005). These comparisons are not restricted to the same species, but also to equivalent niche species. The effects OCs have on birds include reduced reproductive success caused by endocrine disruption, eggshell thinning, embryo mortality, and abnormal reproductive behaviour, to name but a few (Aurigi *et al*., 2000; Dawson, 2000). Exposure to POPs, even at low levels, may constitute a stressor that in combination with other environmental and anthropogenic factors may adversely affect bird populations (Gill & Elliot, 2003; Sakellarides *et al*., 2006; Letcher *et al*., 2010).

Bird eggs are considered good indicators of organohalogen compounds in the environment owing to their high lipid content (Van den Steen *et al*., 2006). During egg formation, lipophilic pollutants are transferred from the female bird to her eggs, thus reflecting the body burden of the female bird (Verreault *et al*., 2006; Braune, 2007), while simultaneously indicating the level of these pollutants in the environment and the exposure of the embryo. However, the concentrations of POPs measured in birds' eggs can be affected by multiple physiological and

environmental factors. These factors include: variations in environmental input, the amount of lipids used during the laying season (Ewins *et al*., 1999), species-specific differences in the amount of OCs conveyed from the female birds to the eggs (Aurigi *et al*., 2000; Van den Steen *et al*., 2009a), trophic position (Borgå *et al*., 2005), clutch size and laying order (Pastor *et al*., 1995).

Eggs are ideal monitoring tools (Medvedev & Markove, 1995; Lebedev *et al*., 1998) since eggs:

- have a fairly consistent composition and decompose slowly,
- are produced by a specific portion of the population,
- are easy to handle and sampling is relatively fast and cost effective,
- sampling is a non-invasive sampling method,
- represent pollutant uptake by the female bird in a period before the egg is laid, and
- allow for random sampling.

The use of eggs as an organism indicator has also been extended to emerging organic pollutants such as brominated flame retardants (BFRs) (Jaspers *et al*., 2005; Polder *et al*., 2008a; Covaci *et al*., 2009). BFRs such as PBDEs are used in combustible material to prevent flames and fire. These chemicals are structurally similar to PCBs, therefore, a similar mode of toxicity is expected (Darnerud *et al*., 2001). Although the exact mode of PBDE toxicity has not been elucidated, PBDEs have been linked to effects on neuro-behavioural development, thyroid hormone homeostasis as well as other endocrine disrupting effects (Darnerud *et al*., 2001; Costa *et al*., 2008). BFRs have been found in various environmental matrices, and widely distributed throughout the world. Although levels of BFRs have previously been measured in birds' eggs from South Africa, it is important to monitor levels and look at various industrial areas and types of industries that may contribute to environmental loadings.

1.2. PROJECT OBJECTIVES

To assess the current levels of POPs in the environment, wild terrestrial and aquatic birds' eggs as well as backyard chicken eggs were analysed for BFRs, OCPs and PCBs. The levels found in chicken eggs were then used in a preliminary risk assessment of humans exposed to these eggs. The Gauteng Province (refer to Figure 12) is a highly industrialised area with chemical production facilities, manufacturing of household goods, as well as a large iron and steel producer. Although located in the Free State Province, Sasolburg and Parys were also included within this study. Sasolburg is home to a large petrochemical plant, with Parys less than 50 km from Sasolburg, housing large breeding colonies of aquatic birds. Many of the

chemicals listed as POPs or emerging POPs are formed as unintentional by-products in a variety of industrial processes. For this reason relative high concentrations of these chemicals were expected in these areas.

To achieve the above, the following objectives were set:

- Establishing the presence and levels of POPs in bird eggs. Chemicals selected for investigation include: PBDEs, hexabromocyclododecane (HBCD), 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), and its major metabolites 1,1-dichloro-2,2(*p*-chlorophenyl)ethylene (DDE) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD), hexachlorocyclohexane (HCH), chlordanes, mirex, hexachlorobenzene (HCB), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and PCBs.
- Assessing important health aspects and the status of the selected ecosystems using the information derived.
- Investigating the distribution and congener profiles/patterns of the different POPs.
    - Determining the influence of trophic level, habitat usage and species specific differences on the congener profiles of selected POPs
    - Investigating differences between the southern and northern hemisphere
    - Assessing the effect of industrial activity and the impact on the region
- Obtaining a preliminary risk assessment of POPs for wild bird populations in South Africa.
- Assessing human exposure and risk to POPs through the consumption of backyard chicken eggs.
- Producing data that can be used for the SC and other relevant conventions and treaties concerning POPs.
- Identifying avenues for further research.

2 Literature review

Due to their tendency to undergo long-range transport and cause toxic effects, use and manufacturing of many legacy POPs are banned or controlled on global and regional scales through international agreements such as the SC (Bidleman *et al*., 2004). Long-range transport occurs through two main pathways namely the one-hop and multi-hop (grasshopper effect) pathways. The one-hop pathway describes the movement of low volatility pollutants such as metals where transport occurs, and following deposition, the pollutant cannot re-enter the atmosphere. The multi-hop process in contrast, describes the motility of semi-volatility pollutants such as most OCs that can re-enter the atmosphere through re-volatilisation (Mcdonald *et al*., 2000). Consequently these chemicals can be deposited far from their origins. As mentioned previously, South Africa, as a signatory of the SC, has a responsibility to undertake research pertaining to POPs. The SC focuses on eliminating or reducing the release of POPs into the environment and came into force on 24 May 2004 (Stockholm Convention on POPs, 2010). Chemicals listed in the SC share a number of characteristics that initially made them ideal for application in industry or for use as insecticides and pesticides. These properties, however, also meant that these chemicals are hydrophobic, lipophilic, semi-volatile, and therefore susceptible to long-range transport, resistant to degradation (persistent), and prone to bio-accumulation (Godduhn & Duffy, 2003). These properties in turn are linked to a variety of toxic responses POPs have in humans as well as wildlife. POPs have been linked to various forms of cancer as well as damage to the nervous-, reproductive- and immune systems (United Nations Environmental Program (UNEP), 2002).

Originally the SC focused on 12 chemicals, the so-called dirty dozen. The dirty dozen comprises PCBs, PCDDs, PCDFs, aldrin, dieldrin, DDT, endrin, chlordane, HCB, mirex, toxaphene and heptachlor (UNEP, 2002). Nine new POPs were added to the SC in May 2009. They are chlordecone, hexabromobiphenyl, hexabromodiphenyl ether (hexaBDE), tetra-bromodiphenyl ether (tetraBDE), pentabromodiphenyl ether (pentaBDE) heptabromodiphenyl ether (heptaBDE), HCH (including α-HCH, β-HCH and γ-HCH), pentachlorobenzene (PeCB), perfluorooctane sulfonic acid (PFOS) and perfluorooctane sulfanyl fluoride (PFOSF) (Stockholm Convention on POPs, 2010).

In an attempt to contribute to current knowledge pertaining to POPs in the South African environment, the two main classes studied during this project included OC contaminants and

the newly added BFRs. The relevant chemicals will be discussed further, in lieu of the interactions between the chemical and physical characteristics of the compound and their eventual environmental fate. These characteristics are also an important factor in the dispersion of the chemical through the food web and can be related to the trophic levels of the bird species studied as well as possible exposure scenarios.

2.1. BFRs

BFRs have been produced since the 1970s, with production in 1999-2000 reaching levels greater than 200 000 metric tons (Birnbaum & Staskal, 2004; De Wit, 2002). There are five classes of BFRs: tetrabromobisphenol A (TBBPA), HBCD, and three commercial mixtures of PBDEs (penta-, octa- and deca-BDEs) (Birnbaum & Staskal, 2004; Wu *et al*., 2010). Although polybrominated flame retardants are an important class of low-cost flame retardants that led to the reduction of fires and consequently a reduction in the number of fire-related deaths in the last few decades, they are also widespread environmental pollutants (Stoker *et al*., 2005; Costa *et al*., 2008). PBDEs are used as flame retardants in ready-made plastic products, textiles, construction materials and electronic equipment (Jaspers *et al*., 2005; Stoker *et al*., 2005) and have been measured in a vast range of environmental matrices including water, soil, air as well as animal and human tissue and breast milk (Costa *et al*., 2008; Polder *et al*., 2008b). Additionally, BFRs have attracted scientific scrutiny not only due to their high frequency of use, but also because of their persistence and hydrophobicity that could indicate a predisposition to bio-accumulation and bio-magnification (Sánchez-Prado *et al*., 2005). Due to these properties tetra-, penta-, hexa-, and hepta-BDE were added to the SC's list of POPs in May 2009. According to EU regulation EC1907/2006, Annex XVII on the Registration, Evaluation and Authorisation of Chemicals (REACH) no product imported into the European Union (EU) may contain more than 0.1% of penta-or octa-BDE per weight basis (Regulation (European Commission (EC)) No 1907/2006). Additionally from July 2006 onwards no new electronic equipment placed onto the market may contain PBDE with the exception of deca-BDE (Directive 2002/95/EC). Deca-BDE was officially banned in the EU from 2008 (De Wit *et al*., 2010).

Although the production and use of PBDEs are now regulated in the EU, these chemicals can still be released into the environment through contaminated sediment and soil reservoirs, in-service products, products produced from recycled materials, as well as the possible debromination of deca-BDE (La Guardia *et al*., 2006). This necessitates the on-going monitoring of environmental levels of BFRs.

2.1.1 Production and use

The PBDE group of BFRs consists of 209 possible congeners (Table 1) (De Wit, 2002), with a basic structure, similar to that of PCBs, as illustrated in Figure 1. Three major commercial formulations of PBDEs are produced through brominating diphenyl ether: deca-BDE, octa-BDE and penta-BDE (De Wit, 2002). The penta-BDE mixture predominantly consist of BDE-99, -47, -100, -153 and -154 (Stoker *et al*., 2005), the octa-BDE of BDE-183, -153, -154, and -209, whereas deca-BDE consist of BDE-209 (De Wit *et al*., 2010). Penta-BDE was used in polyurethane foam in furniture and in adhesives, octa-BDE was used in hard plastics such as computers, and deca-BDE is still used in furniture, electronics, wiring, transportation, and building material (De Wit *et al*., 2010). The most frequently used brominated flame retardant is deca-BDE that is still produced in the United States.

Another major BFR is HBCD. HBCD is a brominated aliphatic cyclic hydrocarbon, formed through the bromination of cyclododecane (De Wit, 2002; De Wit *et al*., 2010) that is primarily used in polystyrene foams and as a replacement for PBDEs in a wide variety of products (Covaci *et al*., 2006). Commercial HBCD (Figure 1) consists of a mixture of five isomers α-, β-, γ-, δ- and ε-HBCD. In most commercial mixtures, γ-HBCD is the predominant isomer (75 - 89%), followed by α- and β-HBCD, with trace amounts of δ- and ε-HBCD (Covaci *et al*., 2006) whereas α-HBCD is the predominant isomer in biota (Isobe *et al*., 2007; Isobe *et al*., 2009). With the restrictions and bans placed on PBDEs, the production and use of HBCD have steadily been increasing (Ueno *et al*., 2006).

Table 1: Name and number of bromines in the investigated PBDEs (La Guardia *et al.,* 2006).

Code BDE	IUPAC name	Nr of bromines
28	2,4,4'-tri-BDE	3
47	2,2',4,4'-tetra-BDE	4
99	2,2',4,4',5-penta-BDE	5
100	2,2',4,4',6-penta-BDE	5
153	2,2',4,4',5,5'-penta-BDE	6
154	2,2',4,4',5,6'-penta-BDE	6
183	2,2',3,4,4',5',6-hepta-BDE	7
206	2,2',3,3',4,4',5,5',6-nona-BDE	9
207	2,2',3,3',4,4',5,6,6'-nona-BDE	9
208	2,2',3,3',4,5,5',6,6'-nona-BDE	9
209	2,2',3,3',4,4',5,5',6,6'-deca-BDE	10

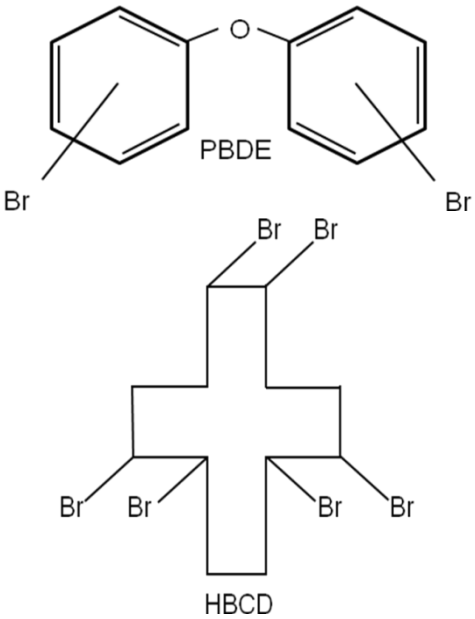


Figure 1: The chemical structures of PBDEs and HBCD adapted from De Wit (2002).

2.1.2 Environmental fate of PBDE and HBCD

PBDEs have a high boiling point, low vapour pressure and are highly hydrophobic with a correspondingly low water solubility (Table 2), indicating both persistence and a high potential for bio-accumulation. BFRs enter the environment through a number of routes (Figure 2). Since, BFRs are often additives and not chemically bound, they easily leach from commercial products. Even when chemically bound, incomplete polymerisation can lead to environmental release (De Wit *et al*., 2002). After production, BFRs are released from BFR-containing products throughout their lifetimes, during use as well as following disposal (De Wit *et al*., 2010). Since BFR-treated products are disseminated globally through trade (Hale *et al*., 2006), these chemicals are measured in the environment of countries such as South Africa where BFRs have never been produced. Consequently they have been measured in various environmental matrices (Polder *et al*., 2008a; Odunsanya *et al*., 2009).

Table 2: Chemical and physical properties of selected BFRs*

Brominated flame retardant	Abbreviation	Formula	Molecular mass	Vapour pressure (Pa)	Melting point (ºC)	Boiling point (ºC)	Water solubility (µg/ℓ)	Log K_{ow}
Tetrabromodiphenyl ether	tetraBDE	$C_{12}H_6Br_4O$	485.8	2.6-3.3 x 10^{-4}	79-82	-	-	5.9–6.2
Pentabromodiphenyl ether	pentaBDE	$C_{12}H_5Br_5O$	564.8	2.9-7.3 x 10^{-5}	92-98	>300	0.0009	6.5–7.9
Hexabromodiphenyl ether	hexaBDE	$C_{12}H_4Br_6O$	643.6	4.2-9.4 x 10^{-6}				6.9–7.9
Octabromodiphenyl ether	octaBDE	$C_{12}H_2Br_8O$	801.5	1.2 – 2.2 x 10^{-7}	~200	-	-	8.4–8.9
Decabromodiphenyl ether	decaBDE	$C_{12}Br_{10}O$	959.2	<10^{-4}	290-306		20-30[#]	10
Hexabromo-cyclododecane	HBCD	$C_{12}H_{18}Br_6$	641.66		175-185		2.1-48.8	5.8

* Darnerud *et al*., 2001; Sjödin *et al*., 2003; Davis *et al*., 2005; [#]Values with a measure of uncertainty

The lower brominated PBDEs have the same long-range transport potential as PCBs and have been detected in the arctic regions (De Wit *et al*., 2010). Although the higher brominated PBDEs have been found in arctic environments they have a greater tendency to bind to particulate matter. The latter seems to be valid for HBCD as well, since HBCD is strongly differentiated regionally, with higher levels generally associated with point sources (Covaci *et al*., 2006). Lower brominated PBDEs are generally more abundant in environmental samples since they are completely absorbed and slowly eliminated leading to bio-accumulation (Sánchez-Prado *et al*., 2005). The most prevalent PBDEs in environmental samples are BDE-

47, BDE-99 and BDE-100 (Sánchez-Prado *et al*., 2005). Although the fully brominated deca-BDE (BDE-209) is poorly absorbed, rapidly eliminated, and is the least bioactive of the PBDEs (Sánchez-Prado *et al*., 2005), research has now shown that it can bio-accumulate, especially in terrestrial food webs (Linberg *et al*., 2004; Park *et al*., 2009). The generalisation can be made that the lower brominated species are prevalent in air and aquatic media as well as biota, while the higher brominated congeners are predominate in atmospheric particulates, soil, sediment and sludge's (Figure 3) (Watanabe & Sakai, 2003; Vonderheide *et al*., 2008). These higher brominated congeners are also more abundant in terrestrial food webs than aquatic food webs when they do occur in biota (Watanabe & Sakai, 2003; Vonderheide *et al*., 2008; Park *et al*., 2009). The higher brominated species also tend to have a longer half-life in soils. The half-life of BDE-28 in soil is greater than 130 days, for BDE-99 greater than 800 days, and for BDE-209 from 300 to 700 days depending on the soil conditions (Scheringer *et al*., 2006; Nyholm *et al*., 2010).

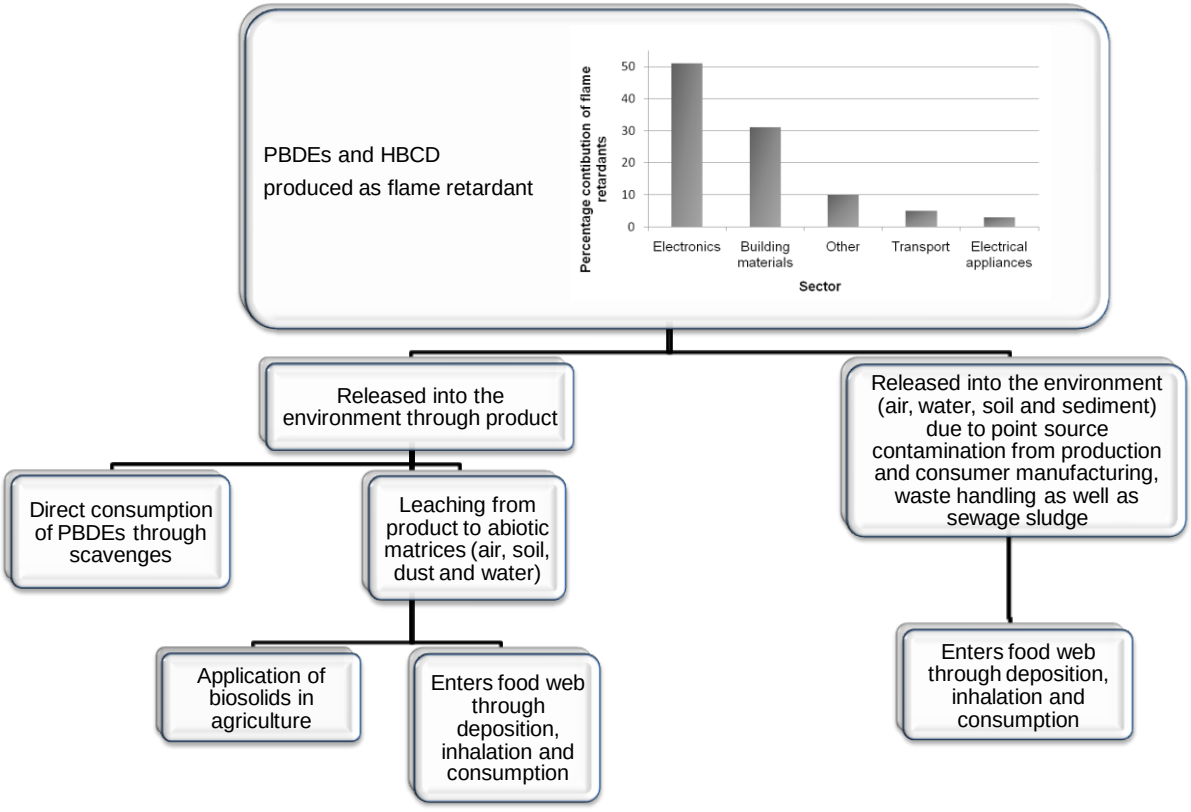


Figure 2: Routes of environmental release for BFRs (adapted from Darneud *et al*., 2001; De Wit, 2002, Thomsen *et al*., 2001; Sjödin *et al*., 2003).

Higher brominated PBDEs can be debrominated in a degree inversely related to the degree of bromination (Sánchez-Prado *et al*., 2005) through photolysis or enzymatic

transformations (Park *et al*., 2009). The extent of photolysis does not seem to depend solely on the degree of bromination, but also on the substitution pattern (Fang *et al*., 2008). PBDEs seem to be susceptible to both hydroxyl radical and photolysis in the atmosphere (De Wit *et al*., 2010). It must be noted that PBDEs can be thermally degraded to brominated dibenzofurans and dibenzodioxins (Stapleton & Baker, 2003). Although biodegradation potential is limited, photo-degradation plays a significant role in the dissipation of PBDEs in water, soils and plants (Sánchez-Prado *et al*, 2005).

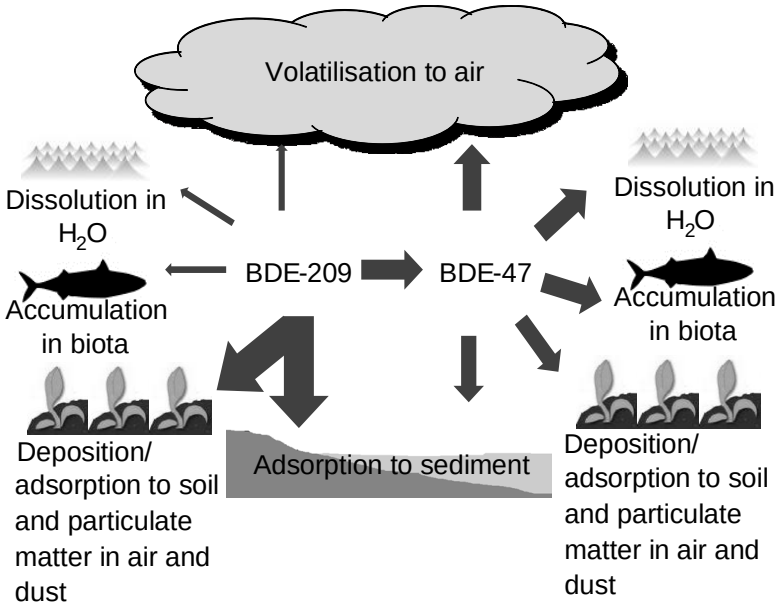


Figure 3: Schematic representation of the variation in behaviour between higher and lower brominated PBDE congeners, adapted from Watanabe & Sakai (2003) and Vonderheide *et al*. (2008).

2.1.3. Toxicology of PBDEs and HBCD

The toxicological effects of PBDEs appear to be similar to those of PCBs, possibly due to the similarities in structure. One of the main effects of PBDEs seems to be on the thyroid hormone. PBDEs affect thyroid levels in the body through two main mechanisms, (1) through competitive binding to the thyroid hormone receptor and (2) altering liver function leading to altered thyroid homeostasis and over elimination of the hormone (Darnerud *et al*., 2001). An impaired thyroid homeostasis and functioning can affect a multitude of physiological parameters including regulation of metabolism, neurological development, sexual maturation and hyperthyroidism (Darnerud *et al*., 2001; Vonderheide *et al*., 2008). Furthermore, research has

shown PBDEs to have a host of other toxicological affects including anti-androgenicity in *in vitro* studies (Stoker *et al*., 2005), interference with sexual development and behaviour, delayed puberty, behavioural and developmental effects that worsen with age, immunotoxicity, toxicity to the kidneys, as well as teratogenicity (Darnerud *et al*., 2001; Vonderheide *et al*., 2008; Park *et al*., 2009). Even at low environmental levels the toxic potential of PBDEs is a cause for concern (Sánchez-Prado *et al*., 2005) since lower PBDE congeners have been linked to carcinogenesis, endocrine disruption, neuro-developmental effects, effects on the reproductive system, liver, thyroid function (McDonald, 2002; Odusanya *et al*., 2009) and induced oxidative stress-mediated neurotoxicity (Tagliaferri *et al*., 2010).

In turn, HBCD exposure can affect the liver and thyroid hormone system. It may cause neurobehavioral alterations (De Wit *et al*., 2010), developmental neurotoxicity, has effects on neurotransmitter uptake, may induce cytochrome P450, and cause non-mutagenic carcinogenesis (Covaci *et al*., 2006). Although the exact mechanism of PBDE and HBCD toxicity is not known, the myriad of toxicological studies on animals do indicate a high toxic potential.

2.2. OC CONTAMINANTS

OCs including PCBs, DDT, HCH, mirex and chlordanes are ubiquitous environmental pollutants that were used extensively for decades before restrictions came into force in the 1970s (Hong *et al*., 2003). DDT, HCH, mirex and chlordanes were used extensively to control various agricultural and horticultural pests and as vector control agents (Kaushik & Kaushik, 2007). PCBs were widely used in industrial applications due to their physical and chemical properties. HCB was produced as a pesticide for industrial application, and is formed as a by-product during industrial and thermal processes. The only group of chemicals that were and still are not actively produced are the dioxin-like chemicals (DLCs) PCDD/Fs. DLCs, including dioxin-like PCBs (DL-PCBs) are formed as by-products of anthropogenic, thermal, chemical and industrial activities. Although many of these chemicals are no longer actively used, OCs are still found in the environment. The highly hydrophobic natures, long half-lives as well as the chemical stability of POPs, lead to their bio-accumulation and bio-magnification in organisms (Hong *et al*., 2003; World Health Organisation (WHO) 2005; McFarland & Clarke, 1989). The chemical properties of these chemicals also determine the rates of deposition, remobilization, long-range transport, as well as the accumulation in water, soil, sediment and biota (Backe *et al*., 2002). The SC has focused attention on the need to identify sources of these chemicals and to continue monitoring for their presence in the environment (Liu *et al*., 2009). From a global

perspective there is still a data gap concerning the occurrence and magnitude of historic/"old generation" or legacy OCs, as well as emerging pollutants in developing countries of tropical and subtropical regions of the world (Bouwman, 2003; Vosloo & Bouwman, 2005). In countries such as South Africa, the use of DDT is still allowed, under Annex B, Part I and II of the SC. According to Part I, OCs can be used for an acceptable purpose or specific exemption and part II states that DDT can be used for the control of disease vectors (Stockholm Convention on POPs, 2010). In South Africa DDT is still utilised in indoor residual spraying (IRS) campaigns in the northeastern parts of the country to combat the disease vector for malaria (Van Dyk *et al*., 2010; Barnhoorn *et al*., 2009).

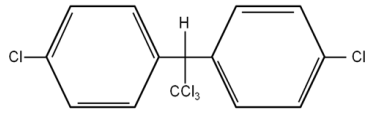
2.2.1. DDT AND ITS MAJOR METABOLITES DDD AND DDE

DDT was first synthesised in 1874 by Othar Ziedler (MacPherson, 1947). However, the insecticidal properties were only discovered in 1939 by the Swiss scientist Paul Herman Müller (Stenerson, 2004; Kaushik & Kaushik, 2007). Initially, DDT was an ideal pesticide due to its physical and chemical properties (Table 3) that led to broad-spectrum effectiveness, stability, persistence, low cost, and low mammalian activity (Kaushik & Kaushik, 2007). DDT was first extensively used as a pesticide during World War II to combat the louse-borne disease typhus and towards the end of the war, malaria. Thereafter, DDT was successfully used from 1947 - 1951 in Northern America to eradicate malaria. The eradication program was less successfully implemented throughout the rest of the world, and in sub-Saharan Africa the programme was never launched (Centre for Disease Control (CDC), 2010). The peak usage of DTT was from the 1950s until restrictions were implemented in the 1970s (Beard *et al*., 2000). At this time evidence started to accumulate concerning the possible long term environmental and health effects linked to DDT (Carson, 2002).

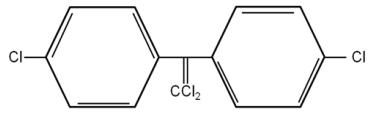
DDT's acute toxicity is caused by the hyper-excitation of the nervous system. Although the exact mode of action has never been elucidated, it is commonly accepted that DDT binds to the nerve membrane and reacts with the voltage-gated sodium channels (Kaushik & Kaushik, 2007; Narahashi *et al*., 2007; Stenerson, 2004). However, due to the structure of DDT (Figure 4) it also affects membrane-linked functions such as oxidative phosphorylation and activity on the axonal membrane by binding with the lipoprotein interface, increasing permeability to sodium ions (Kaushik & Kaushik, 2007).

However, DDT does not only exert acute toxicity. The chemical stability and lipophilicity of DDT and its major metabolites cause these chemicals to accumulate in the food web and are slowly metabolised by most living organisms (Beard, 2006). Within organisms and in the

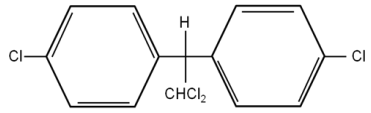
environment DDT degrades into DDE, DDD and 2,2-bis(*p*-chlorophenyl) acetic acid (DDA) (Figure 5). DDT, DDD and DDE have been linked to avian toxicity, including eggshell thinning as early as the 1960s (Ratcliffe, 1970). DDT's effect on eggshell thinning is mainly attributed to endocrine disruption by *p,p'*-DDE as reviewed by Lundholm (1997). Although the exact mechanism of toxicity is not yet known, the exposure of high concentrations of DDE causes numerous effects. These effects include biochemical changes in the calcium metabolism, reduced levels of prostaglandin, calcium, bicarbonate, chloride, sodium, and potassium ions in the eggshell gland during eggshell formation and the disruption of enzymes responsible for the formation of calcium carbonate the main component of avian eggshell (Lundholm, 1997; Berg, *et al*., 2004). These effects then lead to gross morphological changes to the eggshell.These effects then lead to gross morphological changes to the eggshell.



p,p'-DDT



p,p'-DDE



p,p'-DDD

Figure 4: Chemical structure of *p,p'*-DDT, -DDE and –DDD adapted from (ATSDR, 2002a).

Table 3: Physical and chemical properties of *p,p'*-DDT and its metabolites *p,p'*-DDE and *p,p'*-DDD*

Chemical property	*p,p'*-DDT	*p,p'*-DDE	*p,p'*-DDD.
Formula	$C_{14}H_9Cl_5$	$C_{14}H_8Cl_4$	$C_{14}H_{10}Cl_4$
Vapour pressure (Pa at 20-25°C)	2.13×10^{-5}	8×10^{-4}	1.8×10^{-4}
Melting point (ºC)	109	89	109-110
Boiling point (ºC)	Decomposes	336	350
Flash point (ºC)	72.2-77.2	No data	No data
Water solubility (mg/ℓ)	0.025	0.12	0.090
Log K_{ow}	6.91	6.51	6.02
Half-life in soil (years)	2 - 15		

*Agency for Toxic substances and Disease Registry (ATSDR), 2002a; CDC, 2009

The possible endocrine disrupting effect of DDT and its metabolites have been shown for various animal species, as well as in human cohort studies. These effects include endocrine disruption, impacting on reproduction and bone mineral density, carcinogenesis, cardiovascular disease, immune disease and diabetes (Beard, 2006).

Due to the highly persistent nature and potential for long-range transport, DDT, DDE and DDD residues are still found in areas where use had been banned for decades (Hung *et al*., 2007). However, DDT is still actively used for IRS in developing countries where the environmental and health risks posed by DDT are outweighed by the importance of malaria

control. South Africa is one of ten African countries currently using DDT in combating malaria (UNEP. 2010).

The levels of DDT and its metabolites are of particular interest in the South African environment since it is still actively used in the northern areas of South Africa where there are high incidences of malaria. Although DDT is not legally used in any of the current study areas, it is well accepted that DDT can disperse geographically and impact areas far from the initial application (Hung *et al*., 2007). DDT can also originate from the use of dicofol. Dicofol is a non-systemic acaricide produced from technical DDT (Hoekstra *et al*., 2006) used in the control of mites (Clark *et al*., 1990; Qiu *et al*., 2005). Dicofol often contains high levels of DDT as a production impurity and contributes towards more than 70% of the atmospheric DDT measured in certain regions of China (Qui & Zhu, 2010). Dicofol is used in South Africa specifically in fruit cultivation and in domestic gardens (Nel *et al*., 2002).

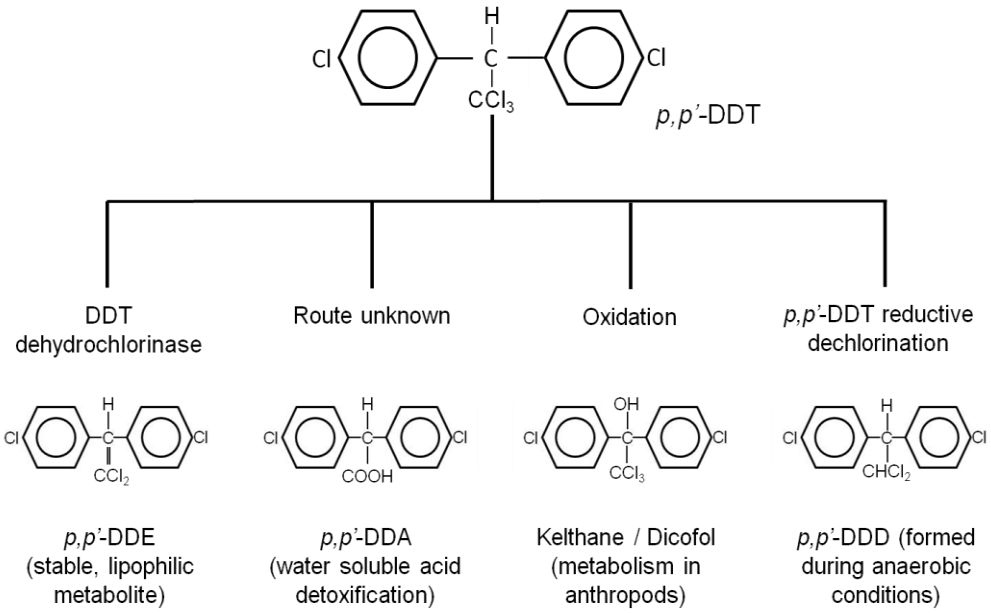


Figure 5: Schematic representation of the major metabolic degradation pathways of *p,p'*-DDT in living organisms (adapted from Walker, 2001).

2.2.2. HCH

γ-HCH (Figure 6), better known as lindane, was one of the most widely used pesticides in the world. The first commercially marketed HCH pesticide, known as technical HCH, consisted primarily of α-HCH (Walker, 2001). Technical HCH contained 55 - 80% α-HCH (Figure 6), 5 – 14% β-HCH (Figure 6) and 8 - 15% γ-HCH (Li *et al*., 2006). After discovering γ-

HCH is the HCH isomer with the most pronounced insecticidal properties (Kaushik & Kaushik, 2007), the technical product was replaced by the more effective lindane that consists of >99% γ-HCH (Walker, 2001).

Lindane is a wide spectrum pesticide that targets synaptic transmission through blocking the gamma-amino butyric acid (GABA)-gated chlorine channels, causing hyper-excitation (Stenersen, 2004). Lindane was commonly used in the treatment of seed, livestock, and timber, as a crop spray, as a household biocide, and for the treatment of ectoparasites (Osibanjo *et al.,* 2002; Walker, 2001) such as head lice and scabies.

The main emission and contamination sources for HCH, are chemical formulation and production plants, disused manufacturing sites, sites used to dispose of HCH, cable manufacturing and smelting of waste polyvinyl chloride (PVC)-coated cables, wood treatment plants, and areas of direct application (Manz *et al*., 2001). Although relatively little literature has been published on the use and production of these chemicals in South Africa, it is known that HCH was produced until the early 1980s at a site in Kempton Park (Osibanjo *et al.,* 2002), Gauteng. In South Africa, lindane is used agriculturally on sunflower, cotton, maize and wheat crops as well as in domestic gardens (Nel *et al*., 2002), indicating the presence of this compound in the South African environment.

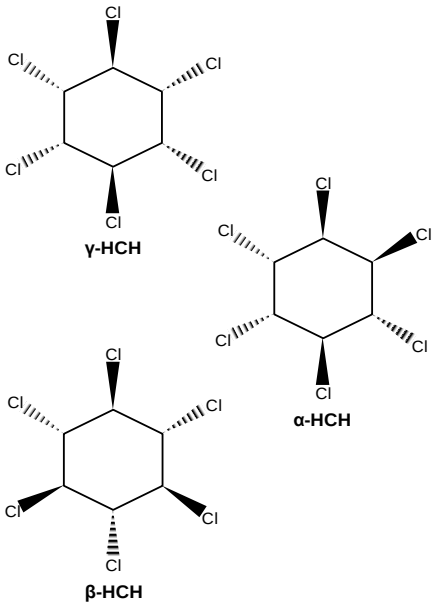


Figure 6: Chemical structure of HCH isomers (ATSDR, 2005a)

Table 4: Physical and chemical properties of HCH isomers*

Chemical property	γ-HCH	α-HCH	β-HCH.
Formula	$C_6H_6Cl_6$	$C_6H_6Cl_6$	$C_6H_6Cl_6$
Vapour pressure (Pa at 20-25°C)	5.5 x10^{-3}	5.9 x10^{-3}	4.8 x10^{-5}
Melting point (ºC)	112.5	159-160	314-315
Boiling point (ºC)	323.4	288	60 (05 mmHg)
Flash point (ºC)	65.6	No data	No data
Water solubility (ppm)	17	10	5
Log K_{ow}	3.72	3.8	3.78
Half-life in soil (d)	14	83	129

*ATSDR, 2005a; Wegmann *et al*., 2007; CDC, 2009

Due to the physical and chemical properties of HCH (Table 4), HCH isomers are highly lipophilic with extended half-lives (Buck *et al*., 1999). In soil, the degradation of HCH depends on conditions such as temperature, pH and organic carbon content (Manz *et al.*, 2001). In the soil compartment, isomerization occurs from γ-HCH to α-HCH to β-HCH (Manz *et al*., 2001). β-HCH is thus often the most prevalent isomer in the environment due to both its increased stability (Wu *et al*., 1997) and the above mentioned isomerization process.

HCH is also toxic. It is known to be a neurostimulant that enhances secretion of serotonin and causes convulsions through excitation of the nervous system, as well as altering the contractile parameters of skeletal myocytes (Buck *et al*., 1999). HCH has also been implicated in the disruption of reproductive function in both male and female animals, specifically rodents. These functions include changes to the male reproduction tract through direct changes to the testis and endocrine regulation, reduced sperm count, effects on sperm motility, spermatogenesis, and decreased serum testosterone levels (Yuksel *et al*., 2009).

On the 9th of May 2009 lindane was added to Annex A, chemicals cited for elimination, of the SC.

2.2.3. CHLORDANE

Chlordane (Figure 7) is an ubiquitous broad spectrum pesticide, produced from the 1940s to the 1980s, for the control of termites and in other agricultural and residential applications until its withdrawal from the world market in 1997 (Dearth & Hites, 1991; Bidleman *et al*., 2004; Hirano *et al*., 2007).

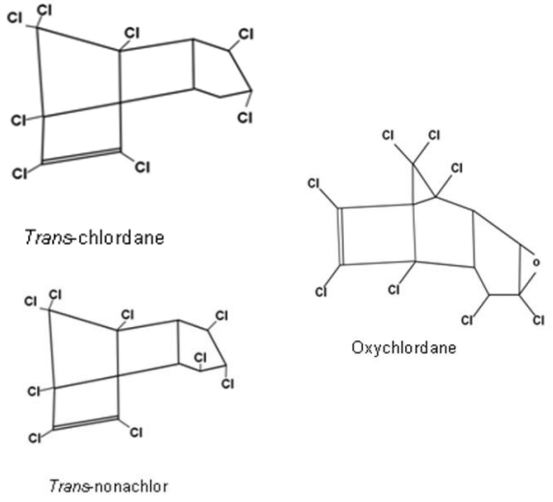


Figure 7: The structure of oxychlordane, *trans*-nonachlor and *trans*-chlordane (adapted from Bondy *et al*., 2003).

Table 5: Physical and chemical properties of chlordanes*

Formula	$C_{10}H_6Cl_8$
Molecular weight	409.8
Vapour pressure (Pa at 25ºC)	0.17
Melting point (ºC)	105-107
Boiling point (ºC) at 0.27 KPa	175
Flash point	56
Water solubility (mg/ℓ)	0.056
Log K_{ow}	5.28 – 8.8
Half-life in soil (years)	5 - 20

Dearth & Hites, 1991; International Programe on Chemical Safety (IPCS), 2000; Bondy *et al*., 2003; ATSDR, 2005b

Chlordane is a non-systemic contact and ingested pesticide classed as a hexachloro-cyclopentadiene pesticide that acts by affecting the GABA neurotransmitter that induces uncontrolled excitations of neurons (IPCS, 2000; Kaushik & Kaushik, 2007). Chlordane is a mixture of over 140 structurally related compounds with the major constitutes *cis*- and *trans*-chlordane, as well as *cis*- and *trans*-nonachlor (Bondy *et al*., 2003; Hirano *et al*., 2007). In organisms, chlordanes are metabolised to mainly less toxic epoxides (IPCS, 2000). Oxychlordane (Figure 7), the major metabolite of chlordane and nonachlors, is however, more toxic and persistent than the parent molecules (Bondy *et al*., 2003). Health effects that have been associated with exposure to chlordanes include stimulation of the nervous system, liver and kidney damage, as well as haemorrhaging of tissue (Janouskova *et al*., 2005).

Since chlordane is no longer actively produced, the main source of the pesticide is from emission of old residues through volatilisation (Bidleman *et al*., 2004). Chlordanes have a very long half-life, resulting in chlordane-related contamination decades after its original application (Dearth & Hites, 1991; Bondy *et al*., 2003). Chlordane's insolubility in water combined with its semi-volatility makes it still detectable in biota and human tissue (Janouskova *et al*., 2005). Additionally, due to the physical and chemical properties of chlordanes (Table 5) it has the ability to undergo long-range transport, to bioaccumulate in the food-web, and to elicit toxic effects (Bidleman *et al*., 2004; Hirano *et al*., 2007). This prompted chlordane's inclusion as one of the original dozen in the SC.

2.2.4. MIREX

Mirex (Figure 8) is a fully chlorinated synthetic compound with a cage-like structure (Kaiser, 1978). It was extensively used from the 1950s as a pesticide and flame retardant (IPCS, 1990) until production was discontinued in 1976 (Comba *et al*., 1993). Concerns were raised regarding the safety of mirex due to its tumour promoting properties, persistence and potential for bio-accumulation in food webs (Kaiser, 1978).

Mirex's physical and chemical properties (Table 6) make it virtually non-biodegradable (CDC, 2009). However, mirex can be degraded to mono-hydromirex products through photolytic degradation and to a small extent reductive dechlorination by anaerobic bacteria (Norstrom *et al*., 1980). Although mirex has never been registered for use as a pesticide in South Africa, it has been detected in bird eggs (Bouwman *et al*., 2008), indicating the possible leaching of mirex from products with mirex as a flame-retardant (Bouwman *et al*., 2008).

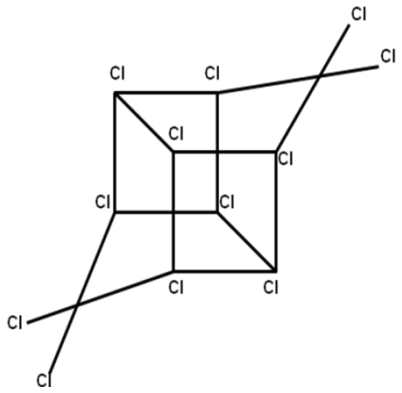


Figure 8: The chemical structure of mirex (IPCS, 1990).

Table 6: Physical and chemical properties of mirex*

Formula	$C_{10}Cl_{12}$
Molecular weight	545.5
Vapour pressure (Pa at 25ºC)	4×10^{-5}
Melting point (ºC)	485
Boiling point (ºC)	No data
Flash point (ºC)	No data
Water solubility (mg/ℓ)	0.2-0.6
Log K_{ow}	5.28 – 8.8
Half-life in soil (years)	12

*IPCS, 1990; ATSDR,1995;CDC, 2009

2.2.5. HCB

HCB (Figure 9) has the distinction of being produced as a pesticide and for a myriad of industrial applications. HCB was first introduced as a fungicide with agricultural use dominating emissions between the 1950s and 1960s (Barber *et al*., 2005). Thereafter, HCB was used as a precursor in the production of herbicides, as an intermediate in dye manufacturing, as a flame retardant, porosity control agent, peptizing agent, fluxing agent, wood preservative, in the production of pyrogenics, ammunition, and as a plasticiser. HCB can also be formed as a by-product of solvent manufacturing and combustion processes (ATSDR, 2002b; Barber *et al*., 2005; Hirano *et al*., 2007). The peak production period for HCB was between the 1970s and the 1980s. During this period, annual worldwide production was approximately 10 000 tons (Hirano *et al*., 2007). Although the production of HCB has declined due to restrictions, HCB is still formed as an unwanted by-product of industrial processes such as pesticide production. It is also released during fuel combustion and waste incineration processes (Liu *et al*., 2009).

The physical and chemical properties of HCB (Table 7), such as its long half-life and relatively low K_{ow}, facilitate long-range transport and bio-accumulation (Bailey, 2001), increasing its environmental recycling (Barber *et al*., 2005). This leads to increased transport potential and wider distributions both locally and globally. HCB has toxic potential with chronic exposure inducing liver tumours and renal and thyroid adenomas in rodents (Michielsen *et al*., 1999). HCB also has the potential to act as an environmental oestrogen and has been linked to human and wildlife health effects including; decreased sperm count, decreased duration of lactation and increases in premature births, as well as congenital malformations (Zheng *et al*., 1999).

However, the major toxic consequence of acute HCB exposure is porphyria characterised by a deficiency in uroporphyrinogen decarboxylase. This has been diagnosed in both humans and birds (Michielsen *et al*., 1999). Symptoms of HCB poisoning in humans include hepatomegaly, enlarged thyroid, splenomegaly, hyper-pigmentation, enlarged lymph nodes, neurological symptoms, painless arthritis and porphyria-independent skin lesions (Michielsen *et al*., 1999).

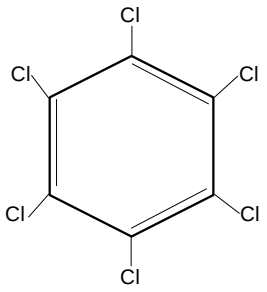


Figure 9: The structure of HCB (adapted from ATSDR, 2002)

Table 7: Physical and chemical properties of HCB*

Formula	C_6Cl_6
Molecular weight	284.79
Vapour pressure (Pa at 25°C)	0.0023
Melting point (ºC)	230-284.78
Boiling point (ºC)	322-326
Flash point (ºC)	242
Water solubility (mg/ℓ)	0.0062
Log K_{ow}	3.9-6.42
Half-life in soil	± 8 years

* ATSDR, 2002b; Jones, 2005

Predatory birds in particular have been used in the bio-monitoring of HCB levels, indicating not only the occurrence of HCB, but also its long-range transport potential and geographical distribution (Barber *et al*., 2005). It is central for successful risk management of industrial or impacted areas that the current levels and possible sources of HCB are known (Bailey, 2001).

2.2.6. PCDDs AND PCDFs

PCDDs and PCDFs (also indicated together as PCDD/Fs) are chlorinated compounds that have similar structures and chemical properties (Figure 10; Table 8). They were also included in the original UNEP dirty dozen (McKay, 2002; Sorgi, 2008) and are now part of the SC. Although PCDD/Fs have never been intentionally produced except for scientific purposes, they are ubiquitous environmental pollutants occurring across the globe in various matrices. Dioxins are highly toxic, causing a myriad of negative health effects such as chloracne, carcinogenicity, hepatotoxicity, teratogenicity, endocrine disruption and alterations in neural development (Poland & Knutson, 1982; Schmitz *et al*., 1994; Sorgi, 2008).

The toxicity of DLC is mediated through the aryl hydrocarbon receptor (AhR). DLCs bind to the AhR and elicit an AhR-mediated biochemical and toxic responses (Behnicsch *et al*., 2001). The AhR is a type II nuclear receptor functioning as a transcription factor (Janošek *et al*., 2006) belonging to the helix-loop-helix group of proteins (Giesy *et al*., 2002). The AhR modulates the response to halogenated aromatic hydrocarbons, polynucleur aromatic hydrocarbons, and phytochemicals such as flavonoids (Mandal, 2005). In the absence of exogenous ligands, the AhR affects metabolism of endobiotics and plays a role in cell cycle regulation (Bock & Köhle, 2005). The binding of DLCs to the AhR-receptor induces phase I and phase II enzymes including cytochrome P450, which is involved in the metabolism of xenobiotics (Rivera *et al*., 2002).

Of the 210 possible PCDD and PCDF congeners, 17 are recognised as toxic (Olie *et al*., 1998), with 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) being the most toxic congener (Table 9). The toxic potential of individual congeners of DLCs are expressed as toxic equivalency factors (TEF) relative to the benchmark congener TCDD which has been assigned a TEF = 1 (Van den Berg *et al.*, 2006). The overall toxicity of a mixture of DLCs, as they occur in nature, is calculated by adding the multiplication of individual congener concentrations to their corresponding TEF value, this value is then known as a TCDD toxic equivalent (TEQ). TEQ values are used for hazard and risk assessments of DLCs, this method however, does assume the additive effect of DLCs (Safe, 1998).

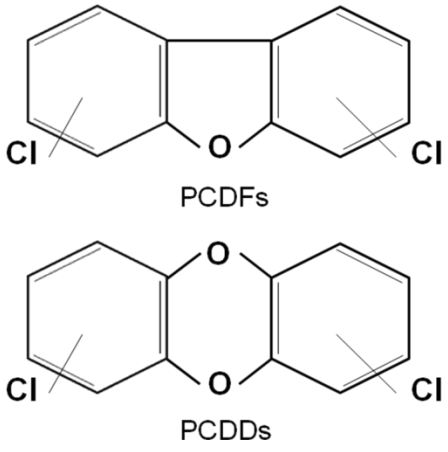


Figure 10: Structure of PCDFs and PCDDs (Behnisch *et al*., 2001)

Table 8: Typical physical and chemical properties of PCDDs and PCDFs*

Formula PCDDs	$C_{12}H_XCl_YO_2$ X= 1-7; Y = 1-8
Formula PCDFs	$C_{12}H_XCl_YO_1$ X= 0-4; Y = 4-8
Vapour pressure (Pa at 20-25ºC)	$0.5 – 1 \times 10^{-10}$
Melting point (ºC)	89 - 330
Boiling point (ºC)	374 537
Flash point (ºC)	No data
Water solubility (mg/ℓ)	$1.2 \times 10^{-4} – 7.4 \times 10^{-8}$
Log K_{ow}	6.2 – 8.8
Half-life in soil	> 10 years

*ATSDR, 1994a; ATSDR, 1994b; Shiu *et al*., 1988; IPCS, 1992; Sinkkonen & Paasivirta, 2000; Walker, 2001; McKay, 2002.

Table 9: WHO/ IPCS TEF-values for PCDDs and PCDFs (Van den Berg *et al*., 2006)

Congener structure PCDD	TEF	Congener structure PCDF	TEF
2,3,7,8-tetrachlorodibenzo-*p*-dioxin	1	2,3,7,8-tetrachlorodibenzofuran	0.1
1,2,3,7,8-pentachlorodibenzo-*p*-dioxin	1	1,2,3,7,8-pentachlorodibenzofuran	0.03
1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin	0.1	2,3,4,7,8-pentachlorodibenzofuran	0.3
1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin	0.1	1,2,3,4,7,8-hexachlorodibenzofuran	0.1
1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin	0.1	1,2,3,6,7,8-hexachlorodibenzofuran	0.1
1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin	0.01	1,2,3,7,8,9-hexachlorodibenzofuran	0.1
Octochlorodibenzo-*p*-dioxin	0.0003	2,3,4,6,7,8-hexachlorodibenzofuran	0.1
		1,2,3,4,6,7,8-heptachlorodibenzofuran	0.01
		1,2,3,6,7,8,9-heptachlorodibenzofuran	0.01
		octachlorodibenzofuran	0.0003

These chemicals are produced naturally through forest fires and volcanoes and through anthropogenic activity as by-products of incineration, pyrogenic and chemical processes that involve chlorine-containing substances (Hays & Aylward, 2003; Raun *et al*., 2005). Dietary uptake is considered to be the major route of non-occupational exposure for DLCs with the consumption of chicken eggs recognised as one of the significant contributors to the daily intake of dioxins (Schuler *et al*., 1997) together with fish, dairy products and meat (Kijlstra, 2004). Chickens are exposed to dioxins through feed, soil, plants, worms and insects (De Vries *et al*., 2006). Due to foraging and geophagy, backyard and free-ranging eggs contain higher levels of dioxins when compared to commercial eggs (Van Ovenmeire *et al*., 2006). Laying hens can excrete between 5 and 30% of their body load of dioxins through the egg (Kijlstra, 2004).

2.2.7. PCBs

PCBs are a class of 209 chlorinated biphenyl congeners that differ in the total number and position of chlorine atoms (Figure 11) situated on the biphenyl molecule (Barron *et al*., 1995). PCBs were introduced into the environment from extensive use in industrial applications due to their chemical inertness, low flammability, and electrical insulating properties (Borgå *et al*., 2005; Jeong *et al*., 2001). Applications included dielectric fluids, lubricants, plasticizers, and paint additives (Takasuga *et al*., 2006). PCBs are ideal for industrial applications because of their chemical and thermal stability. However, these same properties (Table 10) make PCBs environmental pollutants with the ability to accumulate in the environment. PCB congeners degrade very slowly and have the ability to enter the food web (Takasuga *et al*., 2006).

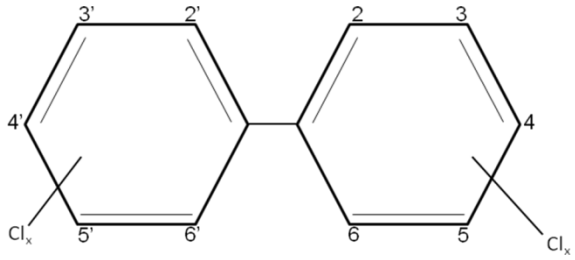


Figure 11: The generic structure of PCBs adapted from Behnisch *et al*. (2001).

Table 10: Physical and chemical properties of PCBs*

Formula	$C_{12}H_{10-x}Cl_x$ x = 1-10
Vapour pressure (Pa)	0.55 – 0.01
Melting point (ºC)	57 – 110
Boiling point (ºC)	275 – 420
Flash point (ºC)	170 - 380
Water solubility (mg/ℓ)	0.0027 – 0.59
Log K_{ow}	4.46 – 8.18
Half-life in soil	> 3 years

*IPCS, 1992; Sinkkonen & Paasivirta, 2000; Gewurtz *et al*., 2006

The hydrophobicity and biological recalcitrance of PCBs result in bio-accumulation in the lipid-rich tissue of biota (Borgå *et al*, 2005). High levels of PCBs in bird populations have been linked to numerous adverse health effects including reduced reproductive potential, developmental and toxic effects such as malformation, pericardial oedema, liver lesions, and lethality to embryos leading to population decreases (Barron *et al*., 1995; Zimmerman *et al*., 1997). Concern over the possible adverse health effects of PCBs led to the restriction of use and eventual ban during the 1970s (Takasuga *et al.,* 2006). However, PCBs are still formed as unwanted by-products of combustion processes in gas-phase or heterogeneous reactions (Lemieux *et al*., 2001) as well as pigment manufacturing (Ishikawa *et al*., 2007). Historic sources of PCBs are also still present in the environment such as electrical equipment, surface coatings, insulating materials, asphalt and waste sites that can contribute to the current PCB loading to the environment through leaching and re-volatilisation processes (UNEP Chemicals, 1999).

PCBs exhibit congener specific toxicity where isosteric congeners exhibit similar biochemical and toxic effects due to similar mechanisms of action. The structural specificity of PCBs for enzyme induction is the most recognised indicator of potential toxicity (McFarland & Clarke, 1989). The presence of vicinal H-atoms (the molecular configuration where any two functional groups are bonded to two adjacent carbon 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 P-450-dependent enzyme systems including mixed-function oxidase (MFO), is responsible for catalysing biotransformation processes of xenobiotics (McFarland & Clarke, 1989; Denison & Whitlock, 1995). PCBs are characterised into three groups each inducing a separate MFO: Phenobarbital-type PCBs (PB-type), 3-

methylchloranthene-type PCBs (3-MC-type) and PCBs that induce both PB and 3-MC known as mixed type PCBs. PB-type PCBs are less toxic and more readily excreted (McFarland & Clarke, 1989). The 3-MC type PCBs are planar molecules with conformational hindrance at the sites for oxygen insertion. This leads to increased stability and decreased detoxification potential. Both, PB- and 3MC-induced enzymatic action can lead to the formation of toxic intermediates (McFarland & Clarke, 1989).

PCBs can thus be classified into five structural groups (Table 11) based on their susceptibility to metabolism by the cytochrome P450 system and the presence or absence of vicinal hydrogen (H) atoms (Boon *et al*., 1997; Borgå *et al*., 2005).

- Group I: The most bio-accumulating PCBs with five to seven chlorine atoms that lack vicinal hydrogen atoms in the *ortho-meta* positions, hindering biotransformation processes through enzymatic activity.
- Group II: Congeners with vicinal H-atoms in the *ortho*- and *meta*-positions with two or more *ortho*-chlorinated substituents.
- Group III: Congeners with vicinal H-atoms in the *ortho*- and *meta*-positions with one or more *ortho*-chlorinated substituents.
- Group IV: Congeners with vicinal H-atoms in the *meta*- and *para*-positions with two or more *ortho*-chlorinated substituents.
- Group V: Congeners with vicinal H-atoms in the *meta*- and *para*-positions with three or more *ortho*-chlorinated substituents.

The most toxic PCB isomers have a similar toxic effect as PCDDs and PCDFs due to their co-planar structure (Lemieux *et al*., 2001). These PCB congeners have chlorine substitutions at the *para* and *meta* positions, with no substitutions on the *ortho* position on the biphenyl ring, giving these molecules the ability to assume a coplanar configuration (McFarland & Clarke, 1989). Chemicals that share this characteristic are known as DLCs. As with PCDD/Fs, coplanar PCBs bind to the AhR and elicit AhR-mediated biochemical and toxic responses (Behnicsch *et al*., 2001). DL-CBs are the most biologically active and have been linked to mutagenesis/carcinogenesis, immunotoxicity and altered endocrine function (Barron *et al*., 1995). In birds, specifically, Ah-mediated toxicity has been suspected to cause reproductive and embryonic effects (Barron *et al*., 1995) including effects on the size and composition of eggs (Fernie *et al*., 2000), PCBs have also been linked to the decline of piscivorous birds (Walker, 2001). In humans, oral exposure has been linked to effects on the cardiovascular system, liver

and skin in the form of abnormal pigmentation and acne (Harris & Jones, 2008). Furthermore, PCBs are listed as probable human carcinogens (Integrated Risk Information System (IRIS), 1997).

The toxicological impacts of the above-mentioned chemicals are of particular concern. These chemicals do not only pose a threat to human health, but also to sensitive animals such as predatory birds.

Table 11: Assignment of PCBs to different metabolic groups, inducer types and structure, as well as WHO/IPCS toxic equivalency factors (TEF) for DL-PCBs*

IUPAC nr	Congener structure	Inducer type[∫]	Group	Structure	TEF mammals	TEF birds*
28	2,4,4'-trichlorobiphenyl		III	Mono-*ortho*		
31	2,4',5-trichlorobiphenyl		VI	Mono-*ortho*		
47	2,2',4,4'-tetrachlorobiphenyl	PB	II	Di-*ortho*		
52	2,2',5,5'-tetrachlorobiphenyl	PB	VI	Di-*ortho*		
56	2,3,3',4'-tetrachlorobiphenyl			Mono-*ortho*		
66	2,3',4,4'-tetrachlorobiphenyl	PB	III	Mono-*ortho*		
74	2,4,4',5-tetrachlorobiphenyl		III	Mono-*ortho*		
77*	3,3',4,4'-tetrachlorobiphenyl	3-MC	III	Non-*ortho*	0.0001	0.05
81*	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	VI	Di-*ortho*		
105*	2,3,3',4,4'-pentachlorobiphenyl	Mixed	III	Mono-*ortho*	0.00003	0.0001
110	2,3,3',4',6-pentachlorobiphenyl		VI	Di-*ortho*		
114*	2,3,4,4'5-pentachlorobiphenyl	Mixed	III	Mono-*ortho*	0.00003	0.001
118*	2,3',4,4',5-pentachlorobiphenyl	Mixed	III	Mono-*ortho*	0.00003	0.00001
123*	2',3,4,4',5-pentachlorobiphenyl	Mixed	III	Mono-*ortho*	0.00003	0.00001
126*	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		VI	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*	2,3,3',4,4',5-hexachlorobiphenyl	Mixed	III	Mono-*ortho*	0.00003	0.0001
157*	2,3,3',4,4',5'-hexachlorobiphenyl	Mixed	III	Mono-*ortho*	0.00003	0.0001
167*	2,3',4,4',5,5'-hexachlorobiphenyl	Mixed	I	Mono-*ortho*	0.00003	0.00001
169*	3,3',4,4',5,5'-hexachlorobiphenyl	3-MC	I	Non-*ortho*	0.03	0.001
170[#]	2,2',3,3',4,4',5-heptachlorobiphenyl	Mixed	I	Di-*ortho*	0.0001	
180[#]	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*	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*		

[#]As listed in the WHO/IUPAC interim toxic equivalency factors; [∫] Inducer types: Phenobarbital-type PCBs (PB-type), 3-methylchloranthene-type PCBs (3-MC) and PCBs that induce both PB and 3-MC (mixed)

*McFarland & Clarke, 1989; Boon *et al*., 1997; Van den Berg *et al.*, 1998; Borgå *et al*., 2005; Van den Berg *et al*., 2006

2.3. BIRDS AS BIOMONITORS OF POPs

Animals inhabiting both terrestrial and aquatic environments are exposed to a wide variety of toxicants from various sources. The same is true for human beings that are exposed not only through their diet, but also through air, soil and water. Research using vertebrates as biomonitoring systems for POPs in the environment provides a link in our understanding of environmental risk to human health and reproductive success (Keithmaleesatti *et al*., 2007). As discussed previously, birds are good biomonitors, with the use of eggs being relatively non-destructive and widely applied in numerous studies (Japsers *et al*., 2005).

It is assumed that a chemical equilibrium exists between hyrdrophobic organic chemicals in eggs and maternal tissue during ovogenesis, due to the transfer of lipoproteins between maternal tissue and the egg (Russel *et al*., 1999). Additionally, within the egg, the influence of metabolism on the concentration of these chemicals is negligible since the enzyme systems are poorly developed in embryonic tissue (Russel *et al*., 1999). Thus, in eggs where the embryo has not developed to any substantial degree, the level of POPs will be similar to the level of POPs in the adult birds at the time of laying. Levels in wild birds' eggs will then be good indicators of the exposure of adult birds to environmental pollutants. If these birds are resident, this indication is of local contamination, whereas migratory birds are good indicators of large-scale scenarios (Chen & Hale, 2010). If birds are categorised into feeding niches and subdivided into terrestrial and aquatic ecosystems, these values can be used to investigate the behavioural differences of POPs in these two environs. The transport and fate (Chen & Hale, 2010) of POPs in the two different environs (terrestrial and aquatic) can thus be elucidated.

The second area where the levels of POPs in bird eggs are of vital importance is the occurrence of POPs in chicken eggs. As mentioned previously, in South Africa backyard poultry is an important source of protein. A preliminary risk assessment to humans can be done, knowing the levels of POPs in both the chickens and their eggs consumed by humans. Furthermore, POPs measured in backyard chicken eggs can be considered good indicators of contamination levels in the area in which the chicken and their owners live (Chang *et al*., 1989).

2.4. SENSITIVITY OF BIRD SPECIES TO ORGANIC TOXICANTS

Due to the widespread use of POPs, as well as their physical and chemical properties such as hydrophobicity that lead to bio-accumulation and bio-magnification, POPs are significant toxicants in various wildlife species (Keithmaleesatti *et al*., 2007). Studies as early as the 1960s directly correlated toxicological impacts of POPs on avian species when this issue was first brought to public attention by Rachel Carson in her book, Silent Spring (Carson, 2002).

Birds are also an ideal bio-monitoring tool indicating the overall health of a specific ecosystem, an example being the eggshell thinning that was attributed to DDE (Blus *et al*., 1997). Since then, more pesticides and industrial chemicals that cause mortality and reproductive impairments in both the embryonic and adult life stages of birds have been identified (Fry, 1995).

One of the main effects of environmental pollutants on health seems to be mediated through endocrine disruption. Endocrine disrupting chemicals (EDCs) can be defined as any chemical that binds to hormone receptors causing the action of the natural ligand to be mimicked or antagonised, chemicals that influence the synthesis or metabolism of hormones, or chemicals that interfere with signalling between components in the endocrine system (Dawson, 2000). EDCs, including DDE, PCDD/Fs and PCBs, have been linked to effects on gonadal steroid hormones (Beard, 2006) and P450 enzymes, altered thyroid hormone function and neuro-endocrine control, as well as activation of the stress response (Fry, 1995; Langer, 1998; Dawson, 2000).

However, these responses to EDCs have never been irrefutably proven in avian species. What is recognized is that PCBs have detrimental effects on wild birds such as increased mortality, immunotoxic effects, impacting bursa development, and decreased growth rates (Hoffman *et al*., 1998; Ottinger *et al*., 2005). DDT and its metabolites are known to cause both lethal and sublethal effects (Fry, 1995) while *p,p*'-DDE has been correlated to eggshell thinning leading to embryo mortality (Lundholm, 1997), delayed sexual maturation and impaired mating behaviour (Ottinger *et al.*, 2005). In the case of PBDEs, the following toxicity in birds has been reported: effects on vitamin status (Murvoll *et al*., 2005), glutathione homeostasis, oxidative stress, immunomodulatory changes, increased growth rates, delayed egg laying, changes in courtship behaviour, decreased egg size, eggshell thinning and reduced reproductive success (Chen *et al*., 2010). Environmental pollutants as a group affects genetic variation in wild bird populations and causes increased oxidative stress (Eeva *et al*., 2006), cause decreased reproductive potential of adults and lead to developmental effects such as malformation and increase embryo mortality (Zimmerman *et al*., 1997), reduce hatchability, cause wasting syndrome, skeletal abnormalities and impair differentiation of reproductive and neural systems (Fry, 2005).

In conclusion, organic compounds, including OCPs (mirex, clordane, lindane, HCB and DDT) and industrial chemicals (PCBs, PCDDs, PCDFs and HCB) have been associated with a variety of behavioural (Fry, 1995; Bustnes *et al*., 2001), developmental and toxic effects in birds (Fry, 1995; Allen & Thompson, 1996), possibly negatively impacting wild populations and

indicating the presence of these contaminants at biologically relevant concentrations. The concentration of these chemicals in specific bird species however, are influenced by exposure levels in the area, the ecology, life history, and metabolic capability of the specific species.

2.5 THE INFLUENCE OF TROPHIC LEVEL AND AVIAN ECOLOGY ON THE CONCENTRATION OF ORGANIC POLLUTANTS

Over the last few decades it has been well documented that organic pollutants, such as PCBs, DDT and HCB, are capable of bio-accumulation and consequent bio-magnification in the food web (Dietz *et al*., 2000*)*. The increase of a chemical's concentration from one trophic level to the next is known as bio-accumulation or trophic transfer (Fisk *et al*., 2001). This pattern has also been demonstrated for emerging POPs such as PBDEs and HBCD that have low levels in terrestrial species when they are compared to marine predators (De Wit *et al*., 2010). The trophic transfer of POPs is related to the complexity and energy flow in the food web (Dietz *et al*., 2000). Homeotherms have greater bio-magnification potential, with bio-magnification in predatory birds higher than in equivalent mammal species utilising the same feeding niche (Fisk *et al*., 2001).

Animal species vary in the degree to which they accumulate POPs due to variations in feeding, digestion, energy allocation (Debryn & Grobas, 2006), metabolic capacities, exposure profiles and the chemical characteristic of the pollutant (Fisk *et al*., 2001). Anthropogenic factors also play a major role in bird ecology. Urbanisation selects a specific host of avian species that have adapted to coexistence with humans (Chace & Walsh, 2006). This in turn impacts the exposure profiles of these species, which can then change the trophic level-POP relationship. Although differences in feeding ecology can explain the majority of variation on pollutant levels among bird species (Behrooz *et al*., 2009), life history also has an important impact on interspecific variation in the concentration and profiles of pollutants (Lavoie *et al*., 2010). Life history parameters that could influence POPs concentrations include age, habitat use (Lavoie *et al*., 2010), clutch size, and when inputs of pollutants are chronic and regular, the longevity and migratory behaviour of a species (Roch *et al.,* 2009).

As mentioned previously, the life history characteristics of birds in warmer climates are different from species in temperate and colder regions. In warmer climates, life history characteristics are typified by small clutch sizes, multiple nesting attempts, long developmental periods, extended parental care (Martin, 1996; Russel *et al*., 2004) and although latitude can play a role on clutch sizes, clutches in the southern hemisphere are generally smaller than those laid at equivalent latitudes in the northern hemisphere (Evans *et al*., 2005). Several

hypotheses have been suggested to explain this phenomenon, including differences in habitat carrying capacity (Cody, 1966), differences in seasonality where reduced seasonality decreases the difference between the size of the breeding populations, environmental carrying capacity, as well as differences in population densities, survival and predation rates (Evans *et al*., 2005). The decreased differences between seasons in the southern hemisphere also impacts migration. Species such as cormorants and egrets that are migratory in the northern hemisphere tend to be non-migratory in South Africa, leading to differences in exposure profiles.

2.6. CURRENT AND HISTORIC LEVELS OF ORGANIC POLLUTANTS IN WILD BIRD POPULATIONS WITHIN SOUTH AFRICA

2.6.1. Historic levels of POPs in wild bird eggs from Southern Africa

Since POPs are highly hydrophobic, they tend to accumulate in the fatty tissue of living organisms, and are toxic to wildlife (Hirano *et al*., 2007), it is important to continually monitor levels in the populations of sensitive species. The first studies of the effects of POPs on avian species in South Africa dates back to the 70s when studies were conducted on the African Fish Eagles (*Haliaeetus vocifer*) (Davies & Randall, 1989). The results of studies pre-dating the 1980s indicate that although contamination problems in South Africa were not as severe as for Europe, DDT levels were in ranges associated with reproductive failure. Greichus *et al*. (1977) reported average DDE and PCB levels in African Darter eggs from the interior of the Cape Province of 34 µg g^{-1} and 1.2 – 2.9 µg g^{-1}dry mass (dm) respectively. De Kock & Randall (1984) reported values of DDE in marine piscivorous bird eggs ranging between 0.004 to 0.92 µg g^{-1} and PCBs ranging between 20 to 106 ng g^{-1} fresh mass (fm). A review by Wiketelius & Edwards (1995) indicated a marked difference in the concentration of pesticides in bird tissue from different feeding guilds within the South African environment. This review also indicated a decrease in the usage of OCPs in Africa as a whole. This review included 1 829 bird samples grouped according to ecological feeding groups' independent of collection site or possible exposure sources. Following these publications, it was a long time before birds eggs were once again monitored for the presence of POPs.

2.6.2. Current state of knowledge for organic pollutants in wild bird populations within South Africa

A previous study, by the North-West University in co-operation with the Norwegian School of Veterinary Science, determined the presence of multiple pollutants in different wild

bird eggs (Table 12) including mirex, oxychlordane, PCBs, PBDE, as well as DDT. This study showed a distinct difference in the congener profile of these chemicals in South African birds when compared to birds from the northern hemisphere, emphasising the need for further research. This study also had the distinction of being the first to report on the levels of BFRs in wild bird populations from South Africa and Africa (Polder *et al*., 2008a).

Limited research has been published during the last 15 years on pesticides in bird eggs form SA, despite the high levels reported previously. In the first study to report on BFR in wild bird populations in SA and Africa, measurable levels of HCB, DDTs HCHs, chordanes and PCBs were found (Table 12) (Polder *et al*, 2008a). This study by the NWU and Norwegian School of Veterinary Science showed a distinct difference in the congener profile of these chemicals in SA birds when compared to birds from the northern hemisphere, emphasising the need for further research. Unexpectedly, mirex was found in all species, although mirex was never registered in South Africa. It also seemed as if terrestrial feeding birds had higher DDE:PCB ratios when compared to aquatic feeding birds (Bouwman *et al*., 2008). The highest ΣOCs levels were measured in the African Darter and the highest level of ΣDDT was found in the Reed Cormorant (Bouwman *et al*., 2008). Both these bird species are predatory birds located high in the food web, feeding on fish, frogs, and the occasional insect.

On the other hand, the African Sacred Ibis eggs contained the highest level of Σ chlordanes. The African Sacred Ibis is an opportunistic feeder, feeding off of waste, small birds and fish and any other food source that can be utilised. The Crowned Plover and Little Grebe occurring lower in the food web, had, on average, lower values.

Table 12: The mean concentration of POPs previously found in selected wild bird eggs collected in South Africa (Polder *et al*., 2008a).

Species	Scientific name	Compound in ng g^{-1} liquid mass (lm)						
		ΣHCH	Oxychlordane	Σ DDT	Σ PCBs	Mirex	Σ PBDEs	HBCD
Cattle Egret*	*Bubulcus ibis*	12	11	331	46	4	2.3	ND
Cattle Egret [#]	*Bubulcus ibis*	12	7	425	122	19	3.7	ND
African Darter[#]	*Anhinga rufa*	1736	153	4430	5070	33	17	3
Reed Cormorant[#]	*Phalacrocorax africanus*	82	56	7033	2657	34	14	ND
African Sacred Ibis[#]	*Threskiornis aethiopicus*	40	230	1176	1048	5	228.5	37.9
Crowned Plover/ Crowned Lapwing[ʃ]	*Vanellus coronatus*	42	6	231	90	6	120	1.6
Little Grebe[l]	*Tachybaptus ruficollis*	16	6	944	197	6	19	ND

Vaal river, * Baberspan, [ʃ] Koppies, ND: no detect

Multivariate analysis of the data clearly distinguished the aquatic from terrestrially feeding birds on the contamination profile. The African Darter (aquatic feeding) and the Cattle Egret (terrestrial feeding) are good representatives of their respective habitats and can therefore be considered as indicator species (Bouwman *et al*., 2008). The article by Bouwman *et al*. (2008) raised the concern that longer living birds in warmer climates, laying fewer eggs per clutch, might be at increased risk when compared to trophically similar birds exposed to equivalent levels of contamination in colder climates.

As can be seen above, all though data for the levels of POPs exist in South Africa, the previous studies were focused on determining the background levels of these compounds in the environment or in specific bird species. Birds were used as indicators due to their sensitivity to POPs as a chemical group. The current study was designed to explore the effects of anthropogenic activity on the presence and distribution of POPs in bird eggs from industrialised areas in South Africa. Therefore, bird roosts and nest were sought in areas where there were high levels of anthropogenic activity. The study sites represented areas with high to low anthropogenic impacts, including natural and agriculturally impacted sites together with residential and industrial areas. Throughout this process the chemical characteristics and the accumulation characteristics of the contaminants were considered. Congener profiles were used to investigate possible sources of pollutants as well as differences between trophic levels. Congener profiles take into consideration both the chemical and physical properties of a pollutant as well as the usage of different congeners in different industrial applications. This was then discussed corresponding to bird ecology (trophic position, feeding habitat and association with humans). The data was then used to determine the possible toxicological effects that these compounds could have on bird populations under, specifically, South African conditions. Furthermore, the present study examined the levels, patterns and sources of industrial pollutants; PCBs, HCB, HBCD and PBDEs, as well as OCPs (HCH, DDTs and chlordanes) in bird eggs from South Africa.

3 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 the Free State Province Department of Tourism, Environmental and Economic Affairs (HK/P1/08760/001) and the Gauteng Provincial Government, Directorate Nature Conservation (CPF6 1340). During the period from October 2008 to January 2009, 77 wild bird eggs were collected from four sampling areas within the Gauteng and Free State provinces. None of the species selected for the project were threatened and all were listed as least concern in the International Union for Conservation of Nature (IUCN) red list (Birdlife International, 2009). All species were non-migratory to ensure as far as possible that exposure was regionally based.

3.1. BIRD SPECIES CHARACTERISATION AND ROUTES OF EXPOSURE

Since the bio-accumulation of POPs in the food web is dependent on exposure related to diet and thus trophic position (Borgå *et al*., 2008; Bouwman *et al*., 2008; Gao *et al*., 2009), bird species were selected to represent four distinct levels in the food web (Table 13). Other factors that can influence the levels and patterns of organohalogenated compounds in birds' eggs include: migratory habits, metabolic capacity, as well as the age of the female birds. To minimise confounding factors concerning routes of exposure, only non-migratory species were selected that are known to be non-migratory or only locally nomadic. The other factors, excluding habitat, were not considered in the scope of the current study. Focus was placed on aquatic birds since the sensitivity of these species to contamination has been previously documented (Guruge *et al*., 1997). Biological information including classification, habitat, diet and breeding habits are given in Table 13. For assessing the influence of trophic level on the concentration of POPs, birds were divided into trophic guilds (Table 13). A guild is defined as a group of species that exploit the same environmental resources (Simberloff & Dayan, 1991) such as food. Granivores are of particular interest since they are often associated with humans. In general, urbanisation selects for omnivore and granivore species, and this coexistence with human's guarantees supplementary food sources (Chace & Walsh, 2006). Due to their position in the food web and elevated exposure in aquatic environments, fish normally have high residues of contaminants in their tissue (Covaci *et al*., 2006). The piscivore avian species feed mainly on fish and are therefore particularly sensitive to POPs.

Table 13: Biological information on bird species sampled (Sinclair *et al*., 1993; Tarboton, 2001; Gibbon & Maclean, 2004; Hockey *et al*., 2005; Birdlife International, 2009).

Common name	Basic appearance	Classification & scientific name	Physical traits	Distribution in South Africa	Habitat and general information	Diet	Breeding habits	Egg description
Black-crowned Night-heron		**Order:** Ciconiiform **Family:** Ardeidae **Species & genus:** *Nycticorax nycticorax* **Species name author:** Linnaeus, 1758	Length 56 cm Diagnostic black crown on the back and backside of neck. Black colouring contrasts grey wings, tail and body.	Widespread in Southern Africa where there is suitable habitat. Absent from arid areas.	Common, locally nomadic, dispersing in response to rainfall. Well vegetated slow moving freshwater water bodies. **Feeding habitat:** Aquatic	**Feeding method:** Nocturnal, crepuscular, hunts singly. Stabs prey. Will swim and dive when feeding. **Main diet:** Diet diverse includes fish, amphibians, reptiles and small mammals. **Guild:** Piscivore	Monogamous colonial breeder, nests alongside herons and egrets. **Nests:** Saucer shaped platforms made from reeds and sticks. Normally in reed beds, trees and cliffs overhanging water at centre or top of colony. **Breeding season:** October – February. **Clutch size**: 2-5	**Appearance:** Rounded oval pale blue green to greenish white. **Size:** 49 x 35 mm
Black-headed Heron		**Order:** Ciconiiform **Family:** Ardeidae **Species & genus:** *Ardea melanocephala* **Species name author:** Vigars and Children, 1826.	Length 96.5 cm Other than the Grey Heron the entire head is black. Yellow marking at eyes.	Widespread. Absent from extremely arid regions.	Common, resident, migrating locally in response to food availability. Distinctively terrestrial in open grassland areas as well as marshes and flooded fields. **Feeding habitat:** Aquatic	**Feeding method:** Solitary hunter. **Main diet:** Terrestrial invertebrates, reptiles, small mammals and birds. **Guild:** Piscivore	Monogamous, colonial or mono-colonial breeder. **Nests:** Large platform of sticks, weeds placed in reed beds or tall trees, cliffs or bushed overhanging water. **Breeding season:** Throughout the year although more commonly in winter. **Clutch size** 2-6	**Appearance:** Oval, pale blue to bluish green. **Size:** 61 x 44 mm
Grey Heron		**Order:** Ciconiiformes **Family:** Ardeidae **Species & genus:** *Ardea cinerea* **Species name author:** Linnaeus, 1758	Length 100 cm White snake-like neck with grey colouring. Black eye plumage with hair like feathers.	Widespread, distribution patchy in Northern Cape.	Moves in response to habitat availability. Prefers shallow water bodies, rarely in dry grasslands. Communal roosts **Feeding habitat:** Terrestrial	**Feeding method:** Diurnal feeder, spears or catches pray. **Main diet:** Fish supplemented by frogs, crabs, insects, rodents, moles and small birds. Food can be taken on wing. **Guild**: Piscivore (Eggs cannot be distinguished from Black-headed Heron)	Monogamous, colonial breeder **Nests:** Large saucer shaped nest of sticks and reeds. Breed in high trees, cliff faces overhanging water or extensive reed beds. **Breeding season:** July – November **Clutch size:** 2-4	**Appearance:** Oval moderately pointed at both ends. Pale blue to bluish green and unmarked. **Size:** 60 x 43 mm.

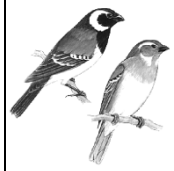
Table 13, continued**:** Biological information on bird species sampled (Sinclair *et al*., 1993; Tarboton, 2001; Gibbon & Maclean, 2004; Hockey *et al*., 2005; Birdlife International, 2009).

African Darter		**Order:** Ciconiiform **Family:** Anhingas **Species & genus:** *Anhinga rufa* **Species name author:** Daudin, 1802	Sharp pointed bill. Long tail and neck with brown/white and black colouring.	Widely distributed throughout South Africa. Distribution restricted to areas with suitable habitat.	Opportunistic local movement. Found mainly in larger rivers and water bodies that are slow moving. Gregarious **Feeding habitat:** Aquatic	**Feeding method:** Dives for food mid-water or along bottom, spears fish. Hunts solitary or in groups **Main diet:** Frogs and fish **Guild:** Piscivore	Colonial nester. **Nest:** Large untidy platform in dead trees/ reeds or on islands. Nest s normally a few meters above water. **Breeding season:** throughout the year. **Clutch size:** 2-7	**Appearance:** Eggs are elongated sub-elliptical, white in colour. Can have brown marks and are smooth. **Size:** 53 x 35 mm
White-breasted Cormorant		**Order:** Ciconiiform **Family:** Phalacroracidae **Species & genus:** *Phalacrocorax lucidus/ Phalacrocorax carbo* **Species name author:** Linnaeus, 1758	White on breast with the rest of plumage glossy-black or brown when not breeding.	Spread along the coast line and widely distributed inland with a patchy distribution in arid regions.	Nomadic in response to changing water levels. Occurs on dams and large stretches of water. **Feeding habitat:** Aquatic	**Feeding method:** Dives for food, but will feed opportunistically. Jaws adapted to catch slow moving benthic fish. **Main diet**: Fish, frogs and crabs. Diet composition linked to prey abundance. **Guild**: Piscivore	Monogamous, colonial neste**r** **Nest: Inland** population Nests in/on trees close to water, protected islands, cliffs and dead trees in recently filled dams. **Breeding season:** March-June **Clutch size:** 3-4	**Appearance:** Sub-elliptical, chalky white with a bluish-green tint. **Size:** 63 x 40mm
African Sacred Ibis		**Order:** Ciconiiform **Family:** Ardeidae **Species & genus:** *Threskiornis aethiopicus* **Species name author:** Latham, 1790	Length 90 cm Bird has white body with characteristic black head. Featherless head and neck with black wing tips.	Common throughout South Africa except arid western coast.	Gregarious, found near water or waste dump sites. Very common in the Rand area. Highly adapted to man-modified habitats and often found on the margins of fresh water wetlands. **Feeding habitat:** Combined	**Feeding method:** Walks slowly talking live prey by pecking and probing mud. Scavenges at refuge tips and abattoirs. **Main diet:** Waste, insects, molluscs, frogs and young birds. **Guild:** Scavenger	Monogamous, colonial, breeder. **Nests:** Slight platform made of twigs, sedge or reeds. Nests in reed beds or trees. Will nest in discrete groups at the fringe of colonies. Nests have been found in abandoned buildings. **Breeding season:** August-December **Clutch size**: 1-5	**Appearance:** Oval, slightly rounded oval. Eggs chalky white, blotched with red/brown spots. **Size:** 66 x 44 mm.

Table 13, continued**:** Biological information on bird species sampled (Sinclair *et al*., 1993; Tarboton, 2001; Gibbon & Maclean, 2004; Hockey *et al*., 2005; Birdlife International, 2009).

Cattle Egret		**Order:** Ciconiiformes **Family:** Ardeidae **Species & genus:** *Bubulcus ibis* **Species name author:** Linnaeus, 1758	Length 54 cm. White plumage, with yellow bill and legs. The legs turn red during the breeding season.	Common throughout South Africa with sparse distribution in dry western regions.	Widely distributed, commonly found in irrigated areas, man-made pastures, among grazing animals, grassland and grassy savannah. **Feeding habitat:** Terrestrial	**Feeding method:** Stabs at prey, either standing still or walking steadily, but can seize prey in air. **Main diet:** insects, fish, amphibians, small rodents and reptiles. **Guild:** Insectivore	Monogamous, colonial breeder. **Nests:** Untidy platforms made of twigs and reeds. Nests in colonies found in trees and reeds close to water. Nest placed lower in colony than other larger species. **Breeding season:** September- February **Clutch size:** 2–7.	**Appearance:** Elliptical, pale green or greenish blue and unmarked. **Size:** 45 x 34 mm
Crowned Lapwing/ Crowned Plover		**Order:** Charadriiformes **Family:** Charadridae **Species & genus:** Vanellus coronatus **Species name author:** Boddaert, 1783	Length: 30 cm Characterised by black grown with white rim and red legs and red colouring on the base of the bill.	Found throughout Southern Africa. Core range is North-West-, Gauteng- and Free State Province	Common, resident with local movement linked to habitat conditions. Normally found in dry open grassland with grass shorter than 60 mm tall. **Feeding habitat:** Terrestrial	**Feeding method:** Visual foragers feeding mainly in the morning and at night in summer and throughout day in winter. **Main diet:** Adult and larval insects mainly feeding on termites. **Guild:** Insectivore	Monogamous, rarely polygamous, solitary nester, territorial. **Nests:** Nest is a scrape lined with pebbles and twigs on broken, gravely or cultivated land. Favours recently burnt or heavily grazed grasslands. **Breeding season:** June-March. **Clutch size:** 2-4	**Appearance:** Pyriform dull olive-brown boldly spotted egg. Spots can be black, grey or white. **Size:** 40 x 29 mm.
Red-knobbed Coot		**Order:** Gruiformes **Family:** Rallidae **Species & genus:** *Fulica cristata* **Species name author:** Gmelin, 1789	Length 43 cm Red knobs on shield well in breeding season. Bird has dark brown to black plumage.	Throughout wetter regions as well as south and east Karoo Generally found in slow moving freshwater bodies.	Common where there are large sheets of water. Often localised in one portion of the lake or dam. **Feeding habitat:** Aquatic	**Feeding method:** Feeds on water surface, dives or crazes on short grasses or cultivated crops on shore. **Main diet:** Plant material, molluscs, crustaceans, insects, scraps, carrion and dung. **Guild:** Omnivore/ insectivore	Monogamous, facultative cooperative breeder, solitary nester. **Nests:** Large cup-shaped nest of reeds/water plants located in shallow water. **Breeding season**: October-November **Clutch size:** 3 - 11	**Appearance:** Oval buff or yellowish stone coloured eggs. Have small round dots of purplish brown. **Size:** 54 x 37 mm.

Table 13, continued**:** Biological information on bird species sampled (Sinclair *et al*., 1993; Tarboton, 2001; Gibbon & Maclean, 2004; Hockey *et al*., 2005; Birdlife International, 2009).

Southern Masked-Weaver / Masked Weaver		**Order:** Passeriformes **Family:** Ploceidae **Species & genus:** *Ploceus velatus* **Species name author:** Veillot, 1819	Length: 17 cm Yellow underside with yellow and black feathers. Dark black mask reaching above red eye.	Near-endemic, widely distributed, scarce only from coastal low lands.	Common: found in almost all habitats where there are trees suitable for nesting. Avoids evergreen forests and coastal bush. **Feeding habitat:** Terrestrial	**Feeding method:** Eats seeds from ground or grass. Also clean insects from substrates such as bark and leaves. **Main diet**: insects, seeds and other plant material. **Guild:** Granivore	Polygamous **Nests:** Kidney shaped with large entrance on underside with a short entrance tube. **Breeding season:** September-January **Clutch size**: 1-6	**Appearance:** oval to elongated. Wide variance in colour. **Size:** 21 x 15 mm
Cape Sparrow		**Order:** Passeriformes **Family:** Passeridae **Species & genus:** *Passer melanurus* **Species name author:** Müller, 1776	Length 15 cm. Males have heavy patterns of black around the head. Females are relatively hard to identify, have grey colouring.	Near-endemic found throughout South Africa with the exception of eastern low-veld.	Common to very common with a preference for dry area as well as drier suburbs of towns and cities. Well adapted to human settlements. **Feeding habitat:** Terrestrial	**Feeding method:** Forages mainly by hopping on ground. Takes fruit and nectar from plants. **Main diet:** Seeds, fruit, insects, the soft shoots of plants and grain. **Guild**: Granivore	Monogamous or polygamous. Nests singly or in loose colonies. **Nests:** Untidy large nests on thorn trees, vines, fences, and telephone poles. **Breeding season:** September–March **Clutch size:** 2 - 6	**Appearance:** white to greenish in colouring with brown flecks. **Size**: 20 x 14 mm
Cape Turtle Dove/ Ring-necked dove		**Order:** Columbiformes **Family:** Columbidae **Species & genus:** *Streptopelia capicola* **Species name author:** Sundevall, 1857	White colouring on tail visible during flight. Has a light grey head and black eyes without the red colouring.	Found throughout South Africa, but generally avoids dense coastal forests.	Common resident that is locally nomadic. Found in most terrestrial habitats including savannas, farmlands, parks and gardens. **Feeding habitat:** Terrestrial	**Feeding method:** Forages on the ground mainly in open patches. **Main diet**: Dry seeds, grains, earthworms and insects. **Guild**: Granivore	Monogamous, territorial solitary breeder. Breeding area permanently occupied. **Nest:** Frail platform of twigs and petioles with a shallow depression lined with grass. **Breeding season:** August - October **Clutch size:** 2	**Appearance:** Oval white eggs with a slightly glossy appearance. **Size:** 28 x 22 mm

3.2. SITE SELECTION

There are a number of factors that can determine the contamination profile in a specific area. Influencing factors impacting on pollutant distribution include the proximity to point sources, amount and frequency of rain, and temperature (Backe *et al*., 2002). The main study area for the current study was the Gauteng Province and Vaal Triangle (Figure 12), which constitutes the industrial centre of South Africa. The sampling area represented various land used, varying from relatively non-impacted to highly impacted areas. The area also represents divergent industries. Industries included in the Vaal Triangle are iron and steel works, electricity generation from coal, petrochemical industries, manufacturing of consumer goods, as well as the historic production of OCPs such as lindane and DDT (Osibanjo *et al*., 2002; Quinn *et al*., 2009). Additionally, Parys, a town located close to Sasolburg, was included in the study area. All of the areas in this study are of particular interest as they support a considerable human population where local resources such as water and home-produced chicken eggs are consumed.

The main area of interest for this study was the Vaal Triangle. The Vaal Triangle is so named as it refers to an area between the three cities of Sasolburg, Vanderbijlpark and Vereeniging that straddles the Vaal River. It is the industrial heartland of South Africa, with approximately 794 600 inhabitants. Sasolburg, located in the Free State, houses one of the largest petrochemical plants in Southern Africa that *inter alia* manufactures catalysts used in hydrocarbon polymerisation for the production of petrol and diesel. This industry also specialises in the production of polymers including styrene and ethylene. Sasolburg is home to chemical and agrochemical manufacturing plants, as well as plastic, including polyethylene (PE), polypropylene and synthetic rubber. Sampling areas (Figure 13) included a wetland on the periphery of the industrial area (Site 5), traffic islands between industries (Site 4) as well as residential areas (Sites 2 and 3). Chicken eggs were collected at the low-income residential areas that are also situated close to industries. The backyard chicken eggs collected in Coalbrook were not only used by the owners, but were also sold at a local kiosk.

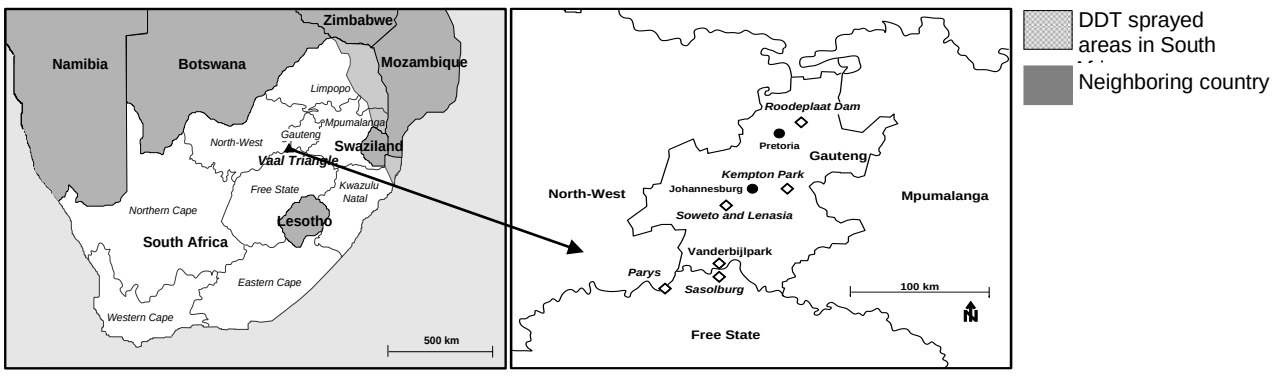


Figure 12: Location of sites where wild bird and chicken eggs were collected.

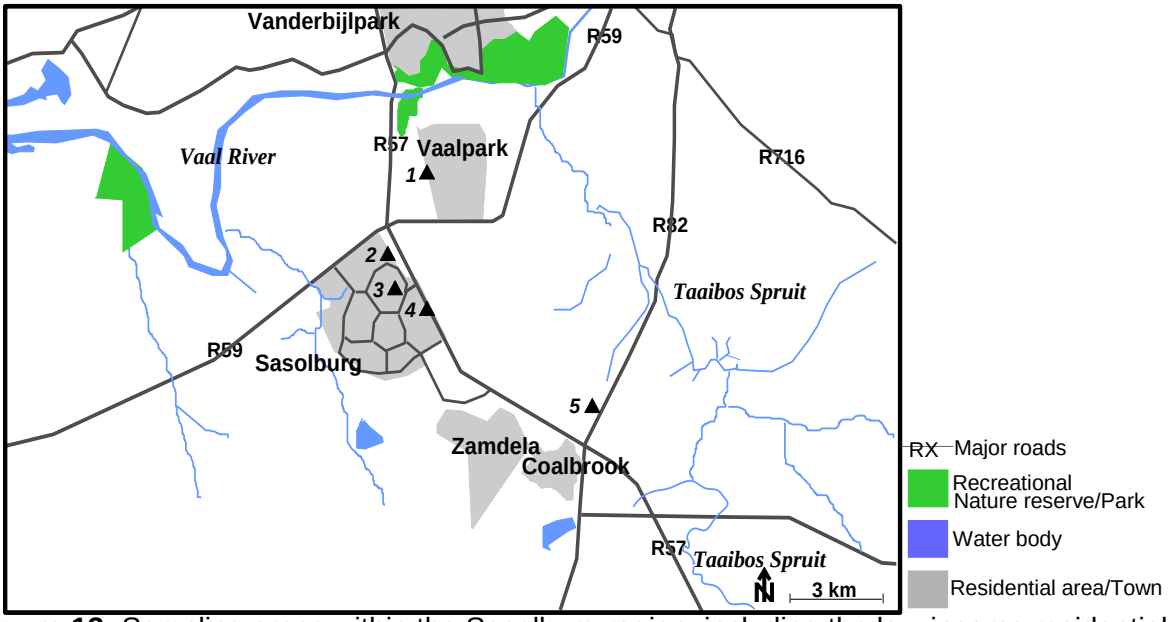


Figure 13: Sampling areas within the Sasolburg region, including the low-income residential areas Coalbrook and Zamdela (see legend Figure 12).

Table 14: Species collection data for the Sasolburg area

Species collected	Site nr	Site description	Nr of samples	Sample nr	GPS co-ordinates	Date
Cattle Egret	5	Wetland system off of the R82	2	$CE_{sb}31$ - $CE_{sb}32$	26º50'08.8" S 27º53'32.2" E	2008/10/20
Cape Turtle Dove	2/3	Residential area	3	$CD_{sb}52$	26º48'10.5" S 27º49'36.7" E	2008/10/20
Crowned Lapwing	3	Residential area	2	$GP_{sb}57$ - $GP_{sb}58$	26º48'10.5" S 27º49'36.7" E	2008/10/20
Crowned Lapwing	1	Vaalpark in open veldt	1	$GP_{vp}56$	26º46'18.5" S 27º50'14.3" E	2008/10/20
Cape Sparrow	4	Industrial area	6	$S_{sb}64$ - $S_{sb}65$	26º48'31.0" S 27º50'13.4" E	2008/10/19

In between the cities of Sasolburg and Vanderbijlpark is a residential area known as Vaalpark. This area has no industrial activity. However, it is located less than 50 km from Sasolburg and Vanderbijlpark. At Site 1, a Crowned Lapwing egg was collected (Table 14). The sampling information for Sasolburg and Vaalpark is summarised in Table 14. Ferrous as well as non-ferrous metal production is known as a source of organohalogen compounds (Choi *et al*., 2008). Vanderbijlpark houses one of the largest steel manufactures in Africa, with low-income residential areas located close to the plant. Vanderbijlpark and Vereeniging are also home to a myriad of other industries including manufactures of steel pipes and wire, including pipes coated with fusion bonded PE and epoxy linings and as electrical wire with insulating materials including PVC and cross-linked polyethylene (XPLE). Furthermore, there are industries producing iron and insulated roofing sheets and heavy-duty equipment including kilns and high power generating equipment.

Terrestrial birds' eggs were collected from residential areas (Sites 3, 4, 9, 8) and from an open field located close to a coal-based power station (Site 5) (Figure 14). Aquatic

samples were collected from Leeukuil Dam (Figure 14). This dam is located adjacent to a low-income residential area. Waste, including sewage, solid household waste and animal offal, are dumped directly into the dam (Site 1 and 2). Eggs of the Southern Masked Weavers were collected in areas close to the Vaal River (Sites 6 and 7). Information on the species and number of eggs collected per site is summarised in Table 15. Apart from the wild bird eggs collected, backyard chicken eggs were also collected from the low-income residential area Sharpeville. In general, the chickens that were kept in this area were in a very poor condition. Open sores and loss of feathers were clearly visible. Battery chicken eggs were procured from different areas in South Africa, including Pretoria (Gauteng); Bloemfontein (Free State) and Stellenbosch (Cape Province) (Figure 12). These eggs were collected to act as reference for backyard eggs. Specifications on the collection of chicken eggs from both Sasolburg and Vanderbijlpark are given in Table 16.

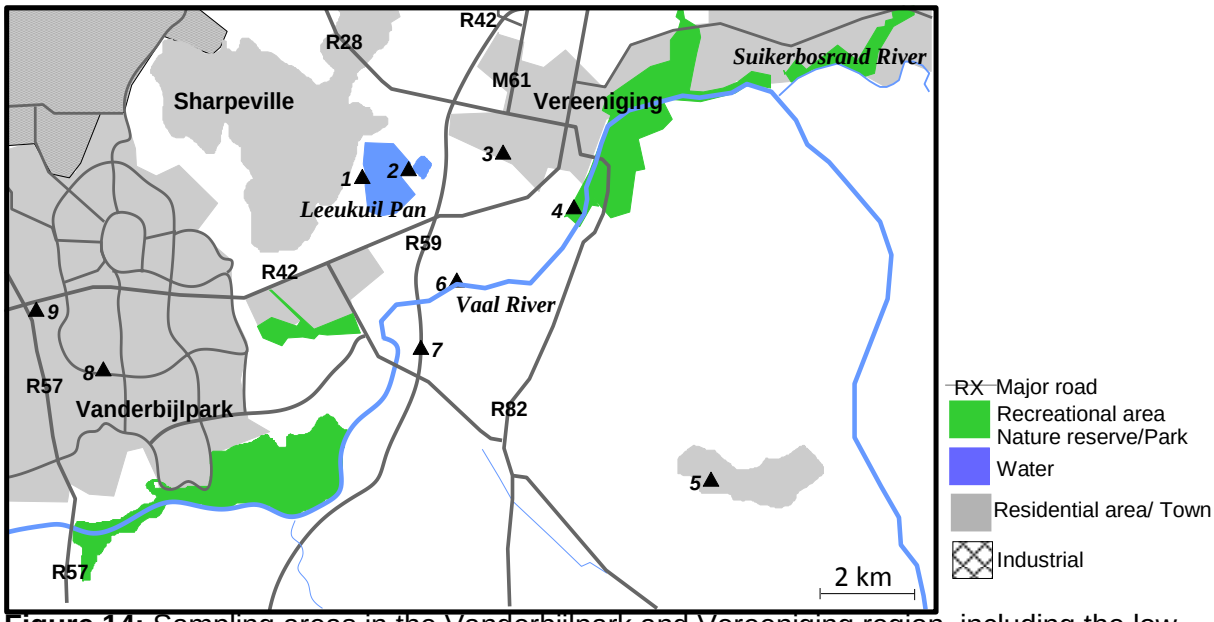


Figure 14: Sampling areas in the Vanderbijlpark and Vereeniging region, including the low-income residential area Sharpeville (see legend Figure 12).

Table 15: Species collection data for the Vanderbijlpark and Vereeniging areas

Species collected	Site nr	Site description	Nr of samples	Sample nr	GPS co-ordinates	Date
Red-knobbed Coot	1/ 2	Leeukop Dam located close to a low-income residential area	2	$RC_{vp}50$ - $RC_{vp}51$	26º41'15.5" S 27º53'35.9" E	2008/10/09
Crowned Lapwing	4	Dickson Park recreational area	1	$CP_{vp}53$	26º41'33.9" S 27º55'55.6" E	2008/10/08
Crowned Lapwing	8	Residential area	1	$CP_{vp}54$	26º42'44.0" S 27º49'17.1" E	2008/10/10
Crowned Lapwing	5	Open field near power station	1	$CP_{vp}55$	26º44'29.4" S 27º57'43.9" E	2008/10/09
Southern Masked Weaver	7	Recreational area on Vaal River	2	$SMW_{vp}61$	26º43'00.6" S 27º54'03.5" E	2008/10/09
Southern Masked Weaver	6	Open veld near Vaal River	2	$SMW_{vp}62$	26º42'12.7" S 27º53'43.0" E	2008/10/08
Cape Sparrow	3,8,9	Various residential areas	3	$S_{vp}66$		2008/10/08

Table 16: Sample collection data for backyard chicken eggs from Sasolburg and Vanderbijlpark, South Africa

Sample	Date	Co-ordinate	Site description	Area
Cbr1 a-c	07/03/2007	S 26º51'39.0" E 27º53'29.0"	Coalbrook (Eggs sold from coop)	Sasolburg
Cbr2 a-c	07/03/2007	S 26º51'25.3" E 27º53'23.5"	Coalbrook (Eggs sold at a tuck shop, very large coop)	Sasolburg
Tsh a-c	12/03/2007	S 26º40'32.8" E 27º51'56.8"	Tshepiso (At the top end of Vanderbijlpark very close to the industrial area, eggs mainly left to hatch).The chickens looked poorly with open sores at the corners of the beaks and raw patches where the feathers have fallen out.	Vanderbijlpark
Shv a-c	12/03/2007	S 26º41'40.4" E 27º52'41.0"	S162 Dubula Drive, Sharpeville. The eggs were also mainly left to hatch and the chickens had raw patches all over there bodies with very few feathers.	Vanderbijlpark

The sampling sites in Parys (Figure 15) consisted mainly of breeding colonies on islands in the Vaal River or along its banks. The Vaal River, the second largest river in South Africa, has its origins east of Johannesburg with most of its catchment upstream of the sampling areas, including the highly industrialised Vaal Triangle as well as agricultural areas surrounding the town of Parys. The Parys sampling sites are approximately 200 km downstream of the Vaal Triangle and 50 km from Sasolburg. The species collected in the Parys area consisted of the Grey and Black-headed Heron, African Darter and the Southern Masked Weaver (Table 17).

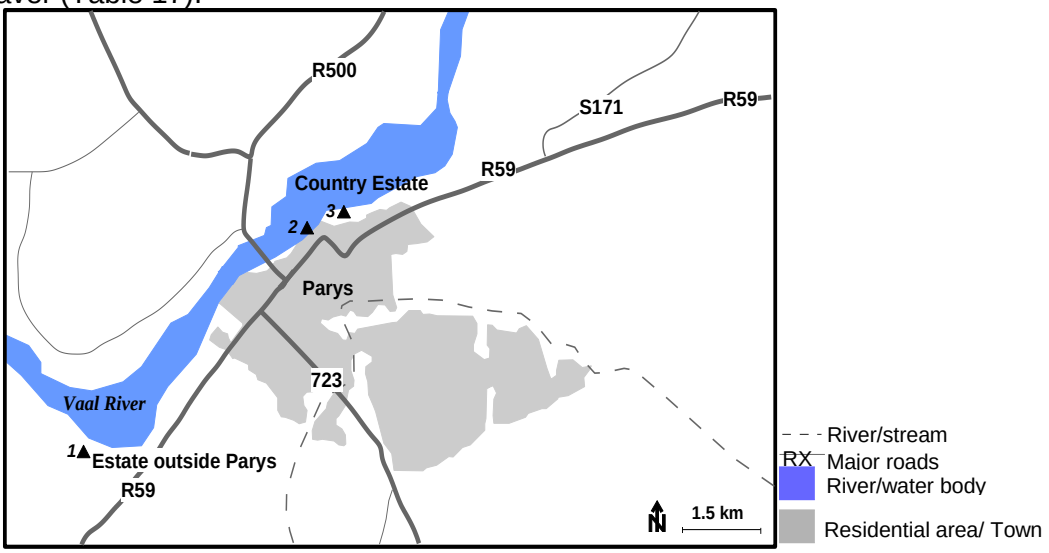


Figure 15: Sampling sites within the Parys region (see legend Figure 12).

Table 17: Species collection data for Parys, Free State Province

Species collected	Site nr	Site description	Nr of samples	Sample nr	GPS co-ordinates	Date
Grey/ Blackheaded Heron	1	Collected on an island in the Vaal River	3	B/GH_p1 - B/GH_p2	26º53'35.9" S 27º27'39.6" E	2008/10/21
Cattle Egret	1	Collected on an island in the Vaal River	2	CE_p29 - CE_p30	26º53'35.9" S 27º27'39.6" E	2008/10/21
African Darter	1	Collected on an island in the Vaal River	5	AD_p34 - AD_p39	26º53'35.9" S 27º27'39.6" E	2008/10/21
Southern Masked Weaver	2	Collected on the banks of the Vaal River	2	SMW_p63	26º53'45.1" S 27º27'14.4" E	2008/10/21
Southern Masked Weaver	3	Collected on the banks of the Vaal River	2	SMW_p60	26º53'35.9" S 27º27'39.6" E	2008/10/21

Wild bird eggs were collected outside the Vaal Triangle from other parts in the Gauteng Province. The three areas sampled were Kempton Park, Soweto, and Pretoria (Figure 16). Kempton Park is a region within the Johannesburg metropolitan area situated approximately 80 km northeast of the Vaal Triangle. It is best known for the largest airport in South Africa. However, in the past Kempton Park housed a site that produced and formulated the OCPs, DDT and HCH (Osibanjo *et al.,* 2002).

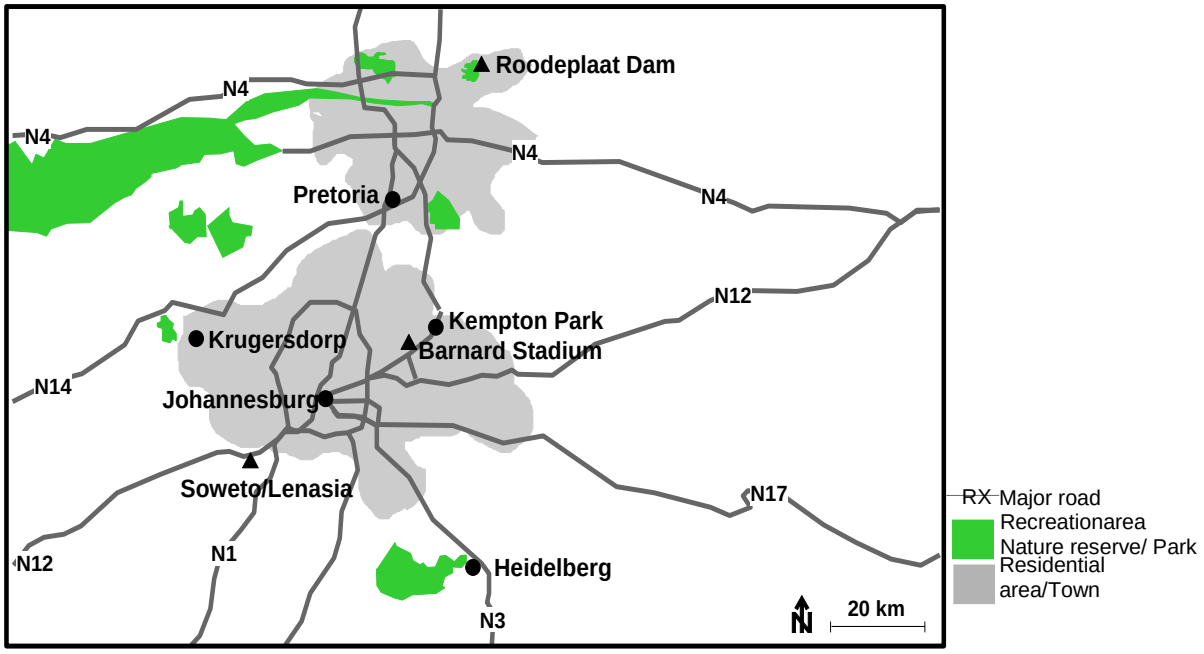


Figure 16: Sampling areas in the Gauteng province including Johannesburg metropolitan area and Pretoria (see legend Figure 12).

Table 18: Species collection data for the greater Gauteng area

Species collected	Location	Site description	Nr of samples	Sample nr	GPS co-ordinates	Date
Sacred Ibis	Soweto/ Lenasia	Wetland	16	SI_s11 - SI_s26		2009/01/15
Black-crowned Night Heron	Soweto/ Lenasia	Wetland	2	BCH_s5 - BCH_s6		2009/01/15
Cattle egret	Soweto/ Lenasia	Wetland	2	CE_s27 - CE_s28		2009/01/15
Red-knobbed coot	Soweto/ Lenasia	Wetland	2	RC_s46 - RC_s47		2009/01/15
Blackheaded heron	Kempton Park	Dam near Barnard Stadium	1	BHH_k4	26º53'35.9" S 27º27'39.6" E	2009/10/24
African darter	Kempton Park	Dam near Barnard Stadium	8	AD_k34 - AD_k35 AD_k40 - AD_k45	26º53'35.9" S 27º27'39.6" E	2009/10/24
Red-knobbed coot	Kempton Park	Dam near Barnard Stadium	2	RC_k48 - RC_k49	26º53'35.9" S 27º27'39.6" E	2009/10/24
White-breasted cormorant	Roodeplaat Dam	Roodeplaat Dam	4	WC_r7 - WC_r10	25º39'11.8" S 28º20'30.4" E	2009/02/03

The birds' eggs collected (Table 18) in Kempton Park were collected from a dam in a residential area that was drying out due to anthropogenic changes upstream in the catchment. Bird's eggs were principally collected from islands in this dam. The Soweto sampling site consists of a wetland system encompassing both Soweto and Lenasia. These

areas are situated outside of Johannesburg and apart from housing a large population there are also industries in the catchment of the wetland. The residential areas range from high to low-income. However, the area directly adjacent to the wetland is in the lower income range. This wetland had large breeding colonies of African Sacred Ibis as well as aquatic bird species (Table 18). The last sampling site within the Gauteng region was Roodeplaat Dam near Pretoria. Roodeplaat Dam is a nature reserve well known for large breeding colonies of cormorants. At this site White-breasted Cormorant eggs were collected (Table 18).

3.3. SAMPLE COLLECTION AND EGG PARAMETER MEASUREMENT

Eggs were hand-collected from nests. Although the laying order of the eggs within the clutches was not known, the smallest egg was collected from each clutch under the assumption that the smallest egg would be the last egg laid. Eggs were wrapped in aluminium foil that had been pre-cleaned with acetone and hexane (Honeywell: Burdick and Jackson™) (US EPA, 1994) and clearly marked. The eggs were transported in a portable freezer and stored -20 ºC in the laboratory of the North-West University Potchefstroom.

Since increasing egg mass and volume are correlated with higher survival (Fernie *et* al., 2000), the mass, width and length of the eggs were measured before the egg content was removed. Each measurement was repeated three times and the mean was used for further statistical analysis. Eggshells were gently rinsed under running tap water to remove the inner membrane and allowed to air dry (Custer *et al*., 1983) for at least three weeks. The thickness of the dried eggshells was measured to the nearest 0.001 mm by the same investigator (following Jaspers *et al*., 2005). Three measurements were taken at the apexes of each egg (Bouwman *et al*., 2008) and each measurement was repeated thrice. The means of these measurements were used in further statistical analysis (Jaspers *et al*., 2005).

3.4. HOMOGENISATION AND TRANSPORT OF EGG SAMPLES

All equipment was pre-cleaned with soap and warm water, rinsed three times with double distilled water followed by a triplicate rinse of 96% ethanol (Saarchem). Eggs were weighed on removal from the freezer and the length and circumference at the widest point were noted (see section 3.3). Each egg was placed in its own glass Petri dish and allowed to thaw, protected from ultraviolet (UV) radiation. Once thawed, the egg contents were carefully transferred from the shell into pre-cleaned high-density polyethylene (HPDE) bottles using a glass funnel and stainless steel utensils. Eggs were homogenised individually, except for Cape Turtle Doves, Cape Sparrow and Southern Masked Weaver that were pooled due to their small egg sizes.

The egg contents, containing both the yolk and albumin, was homogenised with an ultrasonic homogeniser (Misonix sonicator 3000, Farmingdale, NY, USA) taking care to produce as little foam as possible. To prevent contamination the homogeniser was cleaned with warm water and soap followed by rinsing steps with distilled water, ethanol, acetone and hexane. These steps were followed before the first sample and between every following sample. After homogenisation, the HPDE bottles were covered with aluminium foil to avoid exposure to UV light and stored at -20 °C.

The blank controls were two volumes of pre-cleaned glass wool, approximately the size of an average egg that was left out on the bench-top while processing a single egg from thawing to the end of the homogenisation process. This glass wool was therefore exposed to the laboratory environment for the same amount of time as a sample to assess the laboratory environment background levels. The two pieces of glass wool were stored in the same manner as the samples. Additional controls were prepared using double-distilled water that were treated exactly the same as egg samples, underwent homogenisation and was exposed to the same glassware and utensils as the egg homogenates.

The frozen samples and blank controls were couriered to Norway with the necessary export and import permits of Convention on International Trade in Endangered Species (CITES). Upon arrival in Norway it was verified that the samples were still frozen.

The egg homogenates were analysed for: HCB, α-HCH, β-HCH, γ-HCH, chlordanes (oxy-chlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor and *cis*-nonachlor), *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, mirex, 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, -209 and -209) as well as 11 PBDEs congeners (BDE-28, -47, -99, -100, -153, -154, -183, -206, -208 and -209) and HBCD.

3.5. ANALYSIS OF SAMPLES AT NORWEGIAN SCHOOL OF VETERINARY SCIENCE (NVH)

3.5.1. Extraction and clean-up procedures

Sample extraction and gas chromatography (GC) analysis were done in the Laboratory of Environmental Toxicology at the NVH, Norway.

The sample preparation, extraction and clean-up procedures were performed protected from UV-light to avoid the degradation of brominated compounds. The liquid-liquid extraction and clean-up procedures were based on the method of Brevik, 1978; modified as described by Bouwman *et al*. (2009); Polder *et al*. (2008a), and Helgason et al. (2009). Approximately 2 to 3 g of homogenised egg was accurately weighed in an 80 mℓ centrifuge tube and internal standards, 10 mℓ distilled water, 15 mℓ acetone, 20 mℓ cyclohexane and 2

mℓ NaCl (2% solution) were added. The internal standards, for OCs were CB-29, -112 and -207 and, for the BFRs, BDE-77, -119 and -^{13}C-BDE-209 (Cambridge Isotope laboratories Inc., Andover, MA, USA).

The samples were subjected to further ultrasonic homogenisation (4710 Series; Cole Parmer Instruments, Chicago, IL, USA) for 2 min and centrifugation (10 min at 1 643 g). The supernatant consisting of fat and associated hydrophobic organic pollutants 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 lipid determination.

Before GC analysis, sample extracts were treated twice with ultra pure concentrated sulphuric acid (purity 96%: Scanpure, Chemscan AS, Elverum, Norway) to remove any traces of lipids. After sulphuric acid was added to the extract, the extract was mixed with a vortex and placed in a dark area to separate for an hour. These extracts were centrifuged and the supernatant removed. The procedure was repeated with the supernatant. The lipid-free final extracts were concentrated to 0.5 mℓ on a sand bath at 40 °C under a gentle flow of nitrogen gas (99.6%). The final concentrated extracts were transferred to amber GC-vials and sealed for GC analysis.

3.5.2. Lipid determination

Lipid determination was done gravimetrically, up to 4 decimal places. A 1 mℓ aliquot of the sample was placed in pre-weighed glass containers. These extracts were evaporated to dryness in a sand bath until stable weight, and the percentage fat was calculated.

3.5.3. GC analysis

A complete description of the analysis is given in Polder *et al*. (2008a) and Helgason *et al*., (2009). In short, sample analysis for OCs and BFR were performed by high resolution-GC (HR-GC) (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 micro electron capture detectors (Agilent 6890 µ-ECD). Separation was performed with a 1 m long pre-column connected to a dual column system with columns of different polarity and selectivity (SPB-5 and SPB-1701) (60 m, 0.25 mm i.d., 0.25 µm film; Supelco, Bellefonte, PA, USA). The injector temperature was set at 270 °C, 2 µℓ injection volume, pulsed splitless injector mode with a pulse pressure of 414 kPa and pulse time of 1 min, a purge flow of 50 mℓ/min. Hydrogen (H_2) (purity 5.0, Hydro gas, Rjukan Norway) was used as carrier gas with a constant flow of 0.9 mℓ/min, and a make-up gas of 5% methane in argon (Hydro gas, Rjukan Norway) at a constant flow of 0.9 mℓ/min. The temperature

programme used was 90 °C (2 min hold); 25 °C/min increase to 180 °C (2 min hold); 1.5 °C/min increase to 220 °C (2 min hold); and 3 °C/min increase to 275 °C (15 min hold). The total run-time per sample was 70 min.

The separation and detection of mono-*ortho* PCBs (CB-105, -114, -118, -123, -156, -157, -167, and -189) 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 spectrometry (LR-MS) quadrupole detector (Agilent 5973 N) operated in selected ion monitoring (SIM) mode with negative chemical ionization (NCI). The target ions used were as follows: *m/z* 325.8 for CB-112, -123, -118, -115, -105; *m/z* 359.7 for CB-156, -157, -167 and *m/z* 395.7 for CB-189.

Detection of PBDEs (BDE-28, -47, -99, -100, -153, -154, -183), and HBCD were performed by a GC-MS (Agilent 6890 Series; Agilent Technologies) and configured with an MS detector (Agilent 5973 Network; Agilent Technologies). The separation and identification of the compounds were performed on a SB-5 column (30 m, 0.25 mm i.d., 0.25 µm film; Supelco). The PBDEs and HBCD were monitored using NCI in SIM mode at *m/z* 79/81.

For the detection of BDE-206, -208 and -209, 10 mℓ extracts were injected on a GC–MS quadrupole detector (Agilent 5973 network mass-selective detector) equipped with programmable temperature vaporization (PTV) injector (Agilent Technologies). The separation and identification of BDE-206, -208 and -209 were performed on a DB-5-MS column (10 m x 0.25 mm i.d., 10 µm film thickness; J&W Scientific, Agilent Technologies). BDE-206, -208 and -209 were monitored using NCI in SIM mode at mass-to-charge ratio (m/z) 498 and 497.

Chromatographic data was calculated using the software MSD Chemstation (G1701 version C.00.00; Agilent Technologies, Avondale, PA). Due to the poor technical performance on the GC-ECD for the analysis of mirex, the detection limit for mirex was increased by a factor of three. To compensate for positive blank values of CB-209, BDE-183, -206, -207, -208 and -209, the detection limit for these compounds were increased by the average of the blank values plus two standard deviations (Sørmo *et al*., 2009). The data were corrected for the blank values by subtracting the mean blank values plus two standard deviations from each data point. Therefore, CB-209, BDE-183, -206, -207, -208 and -209 results should be considered with caution.

3.5.4. 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 approved through several international inter-calibration tests and CRL EUPT-AO 03 proficiency test on pesticides (HCB and DDTs) in

chicken eggs (2009). As well as WHO, POPs in breast milk (2001-2006); AMAP ring tests for PCBs, OCPs and BFRs in human plasma (2001–2009). The results were all within acceptable ranges.

Quality assurance during analysis included five-point or eight-point linear calibration curves with calculations done in the linear range for each of the analysed standard solutions. Each sample series of between 15 and 20 samples included two recovery samples. Recovery tests were run using homogenised chicken eggs that were spiked with recovery standards. Several solvent blanks and chicken egg blanks were included in each series. The response of the GC was confirmed by running a standard after every ten samples. The reproducibility of the method was tested through analyses of the laboratory's in-house reference material (seal blubber). Limits of quantification (LOQ) for individual compounds were defined as three times the noise level per sample. The LOQs therefore, differed per sample.

3.6. DATA ANALYSIS

All concentrations are given as ng g^{-1} wet mass (wm) except for comparisons with other studies where lipid-normalised values were used on a lipid mass (lm) basis (ng g^{-1} lm). The development stage of the bird embryo strongly influences the lipid content of the bird egg (Speake *et al*., 1998). The eggs collected were not within the same developemental phase. Therefore, statistical analysis was done on a wm basis since it is assumend that the moisture content of the eggs was more stable that the lipid content (Herzke *et al*., 2002). In general, the data used was not corrected for recoveries. However, recoveries are reported in the supplementary data. Data was reported in this manner, to facilitate comparisons with published data. Samples with concentrations below the limits of detection (LOD) were assigned the value of half the LOD, except when specified as no-detects (ND). The substitution of zero for these samples generally leads to an underestimation of the true mean, whereas the substitution of the LOD causes the mean estimated to be an overestimation of the true means (Baccarelli *et al*., 2005). Half the LOD is seen as a compromise between the over and underestimation of ecological data. It must be noted that the statistical treatment of LODs are subjective and depended on the coals of the study. Since statistical analysis was used to determine patterns within the datasets rather than looking at numerical concentration differences between species or individuals, substituting LODs would not make a considerable difference to the outcome of the analysis.

Due to the intrinsic variability of biological data, outliers were not removed from data before statistical analysis. However, when looking at correlations, outliers were removed where appropriate. Statistical analysis was carried out using STATISTICA (version 8) and Canoco for Windows (version 4.5). Furthermore, correlations between different congers,

isomers and pollutant groups were determined. Positive correlations between congeners can indicate similar routes of exposure or mechanisms of accumulation (Stapleton & Barker, 2003).

Variations in concentration were assessed using a one-way analysis of variance (ANOVA) to investigate the differences in concentration and patterns of congeners between the trophic levels (Medvedev & Markova, 1995). If not otherwise specified, all statistical analysis was done on untransformed data. Log transformed data was consistently used when studying patterns and correlation. Log transformations are used to minimise the effect of outliers and elucidate relationships and patterns that could otherwise be masked. When using one-way ANOVA, the sample sizes of groups do not have to be equal and when a statistical difference is identified between the mean of different groups, the post-hoc Tukey test can be used to identify which means are responsible for this difference (Fowler *et al*., 1998). Differences between species were also investigated with basic statistical procedures. Due to the scope of the study, with sample sizes larger than 30, parametric statistical analysis were done assuming a normal distribution of data, with the exception of chicken eggs. Due to the small sample size, normality was tested using the Shapiro-Wilks test. Thereafter, the non-parametric Kruskal Wallis ANOVA and Spearman rank order correlation matrixes were used to analyse the chicken egg data set. The Kruskal Wallis ANOVA compares the medians of three or more samples, where the sample sizes of the groups can be unequal (Fowler *et al*., 2009).

Since principal component analysis (PCAs) provides a visual display, facilitating comparisons (Gao *et al*., 2009) in large data sets, PCAs were also performed. The goal of a PCA is to identify the combination of variables that explain the largest amount of data within a multivariate dataset (Fowler *et al*., 1998). In PCAs, linear combinations of variables are used to explain the variation in a dataset (Sparks *et al*., 1999), where the first principle component explains the largest amount of information in the dataset followed by the second, third, and so forth (Fowler *et al*., 1998). Factor 1 will differ as much as possible from factor 2 so that as much of the data as possible can be explained (Fowler *et al*., 1998). In the current study, PCAs were used to elucidate differences in contamination patterns between species, feeding guilds and geographical locations. Additionally, a second multivariate technique, cluster analysis, was used. Cluster analysis is used to separate variables of a similar kind into groups. Cluster analysis using Euclidean distance measure and the single linkage rule was used to investigate the overall differences in contamination patterns (Goutner *et al*., 2001), when all pollutant groups were considered between species, guilds and habitat usage.

Due to the nature of ecological data, where there can be vast differences in concentration between congeners, a log-ratio transformation was applied to the dataset prior

to the PCA analysis. The use of log-ratio transformation and the advantages thereof is described in Howel (2007). In short, to investigate contamination profiles, the impact of contamination concentration must be minimised so that the variation and relationship between pollutants can be highlighted (Howel, 2007). To address this, 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 $g(p_j) = (p_{j1},\ p_{j2,}\ p_{j3}\ldots\ldots\ldots\ p_{jd})^{1/d}$ PCAs were performed using the software package CANOCO version 4.5.

4 Results

The following results, reported in ng g^{-1} wm except where specified, will be divided into two main sections: (A) levels and patterns of organohalogens in wild bird eggs collected in Gauteng, South Africa and (B) the occurrence of organohalogens in backyard chicken eggs from the Vaal Triangle, South Africa. For statistical analysis and the calculation of the sum of organohalogen groups, ½LOD values were used for NDs. However, if a congener was not quantified in any of the bird eggs analysed, the congener was not considered.

SECTION A
LEVELS AND PATTERNS OF ORGANOHALOGENS IN WILD BIRD EGGS COLLECTED IN SOUTH-AFRICA

4.1 BFRs IN WILD BIRD EGGS

4.1.1. Levels of BFRs

BFRs residues were quantifiable in all eggs analysed; descriptive statistics for the levels of PBDEs and HBCD are summarised in Table 19. The average concentration of BFRs differed statistically significantly between the various bird species (one-way ANOVA $p < 0.0001$). The highest median concentration of ΣBFRs (Table 19) was found in eggs of the African Sacred Ibis (43 ng g^{-1} wm), followed by the Southern Masked Weaver (26 ng g^{-1} wm) and Heron *sp* (13 ng g^{-1} wm). The lowest concentrations of ΣBFRs where measured in eggs of the Crowned Lapwing (2.6 ng g^{-1} wm).

Levels of ΣPBDE ranged from 0.37 – 220 ng g^{-1} wm (Table 19). The highest concentration was found in an African Sacred Ibis egg collected in a Soweto wetland and the lowest concentration was found in a Cattle Egret egg collected in Sasolburg. Levels of HBCD ranged from ND - 9.6 ng g^{-1} wm found in an African Darter egg collected in Kempton Park.

The largest intra-species variation occurred in the Red-knobbed Coot. Values of ΣPBDEs for the Red-knobbed Coot ranged between 6.07 – 208 ng g^{-1} wm (Table 19). The highest value found in the Red-knobbed Coot was in a single individual egg from Soweto with the lowest values measured in eggs collected from Kempton Park (individual concentration in supplementary data, Table S1).

Table 19: BFR concentrations (ng g^{-1} wm) in eggs of various wild bird species

Species	Min	Max	Mean	Median	SD		Min	Max	Mean	Median	SD
Piscivorous species: *African Darter (n = 13)*						**Insectivores/Omnivores : *Cattle Egret (n = 6)***					
%Lipid	1.32	6.45	4.56	5.41	1.65	%Lipid	5.81	8.59	6.89	6.52	1.01
BDE-28	ND	0.18	0.03	0.01	0.05	BDE-28	ND	ND	ND	ND	0.00
BDE-47	0.06	6.48	1.15	0.38	1.98	BDE-47	0.02	0.83	0.16	0.02	0.33
BDE-99	ND	9.74	1.51	0.01	3.63	BDE-99	0.01	7.74	2.91	1.02	3.77
BDE-100	0.07	4.68	1.24	0.41	1.61	BDE-100	0.01	0.39	0.08	0.01	0.15
BDE-153	0.05	2.49	0.52	0.35	0.65	BDE-153	0.03	1.02	0.33	0.12	0.42
BDE-154	0.14	6.07	1.45	0.78	1.68	BDE-154	0.01	1.61	0.37	0.05	0.70
BDE-183	ND	1.00	0.23	0.15	0.29	BDE-183	0.02	0.78	0.26	0.15	0.29
BDE-206	ND	0.91	0.23	0.05	0.31	BDE-206	0.05	0.17	0.07	0.05	0.05
BDE-207	ND	0.23	0.07	0.04	0.08	BDE-207	0.02	0.20	0.06	0.02	0.07
BDE-208	ND	0.13	0.02	0.01	0.03	BDE-208	0.01	0.13	0.04	0.03	0.05
BDE-209	ND	3.16	1.68	1.56	0.44	BDE-209	ND	ND	ND	ND	0.00
ΣBDE	*2.02*	*19.92*	*8.14*	*4.19*	*6.27*	*ΣBDE*	*0.37*	*8.01*	*4.21*	*4.25*	*3.76*
HBCD	0.15	9.60	1.65	0.56	2.92	HBCD	0.15	1.17	0.32	0.15	0.42
ΣBFR	*2.17*	*29.51*	*9.79*	*4.75*	*8.80*	*ΣBFR*	*0.52*	*8.16*	*4.53*	*4.85*	*3.93*
White-breasted Cormorant (n = 4)						***Crowned Lapwing (n = 6)***					
%Lipid	3.80	5.41	4.22	3.83	0.79	%Lipid	8.71	15.51	11.48	11.12	2.21
BDE-28	ND	ND	ND	ND	0.00	BDE-28	ND	ND	ND	ND	0.00
BDE-47	0.42	1.39	0.85	0.81	0.43	BDE-47	0.22	1.29	0.68	0.61	0.40
BDE-99	0.01	0.12	0.07	0.08	0.05	BDE-99	0.31	1.30	0.63	0.43	0.40
BDE-100	0.43	1.08	0.71	0.65	0.31	BDE-100	0.01	0.40	0.16	0.10	0.15
BDE-153	0.46	1.60	0.96	0.89	0.54	BDE-153	0.15	1.00	0.34	0.23	0.32
BDE-154	0.68	2.11	1.29	1.19	0.67	BDE-154	0.06	0.37	0.14	0.10	0.11
BDE-183	0.07	0.15	0.11	0.10	0.04	BDE-183	0.11	1.22	0.38	0.21	0.42
BDE-206	0.05	0.41	0.14	0.05	0.18	BDE-206	0.05	1.33	0.45	0.18	0.54
BDE-207	0.02	0.11	0.04	0.02	0.04	BDE-207	0.07	0.34	0.15	0.11	0.11
BDE-208	ND	ND	ND	ND	0.00	BDE-208	0.01	0.21	0.08	0.05	0.07
BDE-209	1.56	3.19	1.97	1.56	0.81	BDE-209	ND	ND	ND	ND	0.00
ΣBDE	*4.19*	*8.03*	*6.15*	*6.18*	*1.59*	*ΣBDE*	*1.46*	*6.83*	*3.03*	*2.62*	*1.95*
HBCD	0.31	0.96	0.62	0.61	0.32	HBCD	0.15	0.40	0.22	0.15	0.11
ΣBFR	*4.50*	*9.00*	*6.77*	*6.80*	*1.88*	*ΣBFR*	*1.78*	*6.97*	*3.24*	*2.89*	*1.91*
Heron spp (n = 6)						***Red-knobbed Coot (n = 6)***					
%Lipid	5.60	7.26	6.25	6.18	0.62	%Lipid	12.43	14.11	13.28	13.26	0.54
BDE-28	ND	ND	ND	ND	ND	BDE-28	ND	ND	ND	ND	0.00
BDE-47	0.02	0.06	0.04	0.05	0.02	BDE-47	0.09	2.97	1.48	1.42	1.46
BDE-99	0.06	0.37	0.19	0.15	0.12	BDE-99	1.08	9.80	5.19	4.69	4.44
BDE-100	0.01	0.22	0.10	0.09	0.09	BDE-100	0.01	0.48	0.17	0.10	0.20
BDE-153	0.18	22.21	5.46	1.22	8.69	BDE-153	0.03	0.26	0.14	0.13	0.09
BDE-154	0.05	0.67	0.24	0.17	0.23	BDE-154	0.07	0.09	0.08	0.09	0.01
BDE-183	0.09	24.74	6.64	1.56	9.92	BDE-183	0.02	0.20	0.06	0.04	0.07
BDE-206	0.05	1.62	0.45	0.20	0.61	BDE-206	0.05	3.25	0.67	0.12	1.28
BDE-207	0.02	0.64	0.26	0.22	0.25	BDE-207	0.02	1.28	0.30	0.10	0.49
BDE-208	0.01	0.41	0.14	0.09	0.17	BDE-208	0.01	0.56	0.16	0.08	0.21
BDE-209	1.56	16.84	4.11	1.56	6.24	BDE-209	1.56	193.25	33.51	1.56	78.26
ΣBDE	*2.18*	*52.01*	*17.61*	*12.91*	*19.52*	*ΣBDE*	*6.07*	*208.42*	*41.76*	*8.65*	*81.67*
HBCD	0.15	0.61	0.27	0.15	0.20	HBCD	0.15	2.24	0.76	0.37	0.80
ΣBFR	*2.32*	*52.42*	*17.88*	*13.05*	*19.64*	*ΣBFR*	*6.45*	*208.78*	*42.52*	*10.25*	*81.48*
Scavenger species: African *Sacred Ibis (n = 16)*						**Granivores : *Cape Turtle Dove (n = 3: 1 pool of 3 eggs)***					
%Lipid	4.12	7.60	5.83	5.61	0.91	%Lipid	8.52				
BDE-28	ND	ND	ND	ND	ND	BDE-28	ND				
BDE-47	0.11	0.59	0.23	0.16	0.15	BDE-47	ND				
BDE-99	0.25	1.76	0.77	0.58	0.45	BDE-99	10.99				
BDE-100	ND	0.72	0.29	0.27	0.21	BDE-100	0.03				
BDE-153	0.37	50.62	7.26	4.31	11.73	BDE-153	0.08				
BDE-154	0.07	4.51	0.68	0.41	1.04	BDE-154	0.07				
BDE-183	0.43	121.18	18.92	12.10	28.04	BDE-183	ND				
BDE-206	ND	1.79	0.83	0.90	0.40	BDE-206	ND				
BDE-207	0.22	15.74	4.48	4.40	3.54	BDE-207	0.05				
BDE-208	0.20	2.05	1.35	1.52	0.62	BDE-208	ND				
BDE-209	ND	25.48	15.67	15.64	6.92	BDE-209	ND				
ΣBDE	*3.54*	*220.92*	*50.44*	*43.49*	*47.66*	*ΣBDE*	*11.21*				
HBCD	0.92	4.83	2.70	2.75	1.26	HBCD	0.34				
ΣBFR	*4.46*	*222.23*	*53.14*	*45.92*	*47.35*	*ΣBFR*	*11.56*				

ND no detected

Table 19 continued: BFR concentrations (ng g^{-1} wm) in eggs of various wild bird species.

Species	Min	Max	Mean	Median	SD		Min	Max	Mean	Median	SD
Granivores						**Granivores**					
Cape Sparrow (n = 9: 3 pools of 3 eggs)						*Southern Masked Weaver (n = 8: 4 pools of 2 eggs)*					
%Lipid	3.85	7.14	5.84	6.54	1.75	%Lipid	2.97	6.06	4.46	4.40	1.29
BDE-28	ND	ND	ND	ND	0.00	BDE-28	ND	ND	ND	ND	0.00
BDE-47	0.03	31.62	10.57	0.06	18.23	BDE-47	0.39	147.18	39.84	5.89	71.74
BDE-99	10.16	11.44	10.73	10.58	0.65	BDE-99	0.49	15.88	6.82	5.46	7.62
BDE-100	ND	ND	ND	ND	0.00	BDE-100	0.05	0.14	0.09	0.08	0.04
BDE-153	0.09	0.21	0.15	0.17	0.06	BDE-153	0.15	2.28	0.76	0.31	1.01
BDE-154	0.01	0.09	0.06	0.08	0.04	BDE-154	0.03	1.43	0.41	0.08	0.69
BDE-183	0.14	0.17	0.15	0.15	0.02	BDE-183	0.07	1.77	0.57	0.22	0.80
BDE-206*	ND	ND	ND	ND	0.00	BDE-206*	0.05	0.32	0.12	0.05	0.13
BDE-207*	0.06	0.13	0.10	0.12	0.04	BDE-207*	0.08	0.63	0.27	0.19	0.25
BDE-208*	0.07	0.10	0.09	0.09	0.02	BDE-208*	0.06	0.38	0.16	0.11	0.15
BDE-209	ND	ND	ND	ND	0.00	BDE-209	1.56	3.86	2.14	1.56	1.15
Σ*BDE*	*11.16*	*42.44*	*21.82*	*11.86*	*17.86*	Σ*BDE*	*3.46*	*149.81*	*51.17*	*25.71*	*67.07*
HBCD	0.36	0.39	0.37	0.37	0.01	HBCD	0.33	1.28	0.59	0.36	0.47
Σ*BFR*	*11.54*	*42.80*	*22.20*	*12.25*	*17.85*	Σ*BFR*	*4.74*	*150.17*	*51.76*	*26.06*	*66.85*

ND: not detected

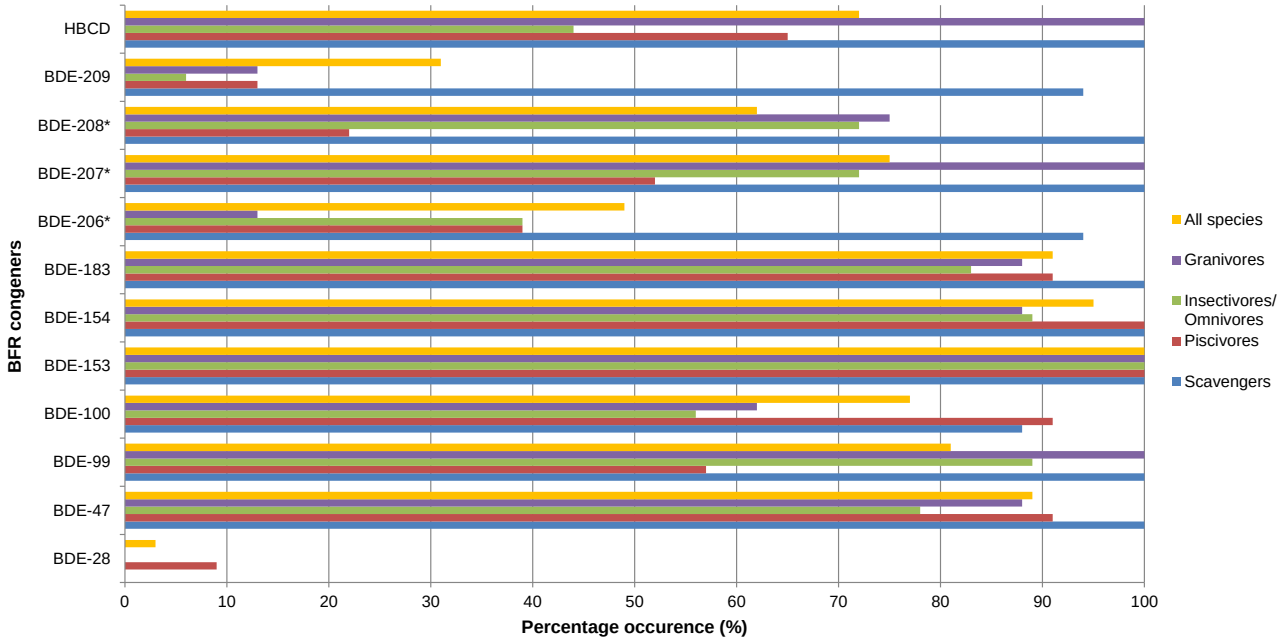
4.1.2 The percentage occurrence of individual PBDE-congeners and HBCD

Amongst the PBDEs analysed, BDE-153 was the most prevalent in wild bird eggs. BDE-153 was quantifiable in 100% of the wild bird eggs sampled, followed by BDE-154 (95%), -183 (91%), and -47 (89%). The PBDE that occurred the least was BDE-28 (3%) followed by BDE-209 (31%; Figure 17). HBCD occurred in 72% of the wild birds analysed. When the occurrence of BFRs were analysed per feeding guild, the following pattern emerged:

- for scavengers BDE-47, -99, -153, -154, -183, -207, -208 and HBCD were quantifiable in 100% of samples;
- for piscivores BDE-153 and BDE-154 were quantifiable in 100% of samples, with HBCD being quantifiable in 65% and BDE-28 in 9% of birds eggs analysed;
- for insectivores and omnivores BDE-153 occurred in 100%, HBCD in 44% and BDE-209 in only 6% of samples; in granivores BDE-99, -153, -207 and HBCD were quantifiable in 100% of wild bird eggs (Figure 17).

The percentage occurrence of individual BFRs in the different bird species analysed is listed in Table 20. All PBDEs excluding BDE-206, -209, and -28 were quantifiable in 100% of the Southern Masked Weaver eggs. In Cape Sparrows BDE-28, -100, -206, and -209 were not quantified in any samples. In contrast BDE-47, -99, -153, -183, -207, and HBCD were present in all samples tested. For the African Sacred Ibis, all BFRs, excluding BDE-28 that was not quantified, occurred in between 88 – 100% of samples. The African Darter was the only species where BDE-28 was measured, and then only in 23% of the samples analysed. All the White-breasted Cormorant samples contained BDE-47, -100, -153, -154, -183, as well as HBCD whereas BDE-28 and -208 were not quantified. For the Heron *spp*.

BDE-99, -153, -154, and -183 were present in all samples with only BDE-28 not quantified in any. The occurrence of other BFR compounds ranged between 17 - 67%. For the Crowned Lapwing BDE-47, -99, -153, -154, -183, and 207 were present in all samples. BDE-28 and 209 were not quantified with the occurrence of other BFRs, varying between 33 - 83%. The Red-knobbed Coot samples all contained BDE-47, -99, -153, and -154; only BDE-28 was not



quantified in any sample and the occurrence of quantifiable BFRs ranged between 17 - 83%.

Figure 17: Composition of BFRs in the different trophic guilds.

Table 20: Percentage occurrence of BFRs in the various bird species analysed

BFR	African Sacred Ibis	Cattle Egret	African Darter	White-breasted Cormorant	Heron *spp*	Crowned Lapwing	Red-knobbed Coot	Cape Sparrow	Southern Masked Weaver
BDE-28	0	0	23	0	0	0	0	0	0
BDE-47	100	33	100	100	67	100	100	100	100
BDE-99	100	67	38	75	100	100	100	100	100
BDE-100	88	33	100	100	67	83	50	0	100
BDE-153	100	100	100	100	100	100	100	100	100
BDE-154	100	67	100	100	100	100	100	67	100
BDE-183	100	83	85	100	100	100	67	100	100
BDE-206	94	17	46	25	50	50	50	0	25
BDE-207	100	33	62	25	67	100	83	100	100
BDE-208	100	67	15	0	50	83	67	67	100
BDE-209	94	0	15	25	17	0	17	0	25
HBCD	100	17	69	100	33	33	83	100	100

4.1.3 Congener profiles of BFRs in individual species

The BFR congener profiles for the eggs collected in the various species are illustrated in Figure 18. As mentioned earlier the levels of BFRs differed statistically

significantly between the various bird species ($p < 0.0001$, one-way ANOVA of log transformed data). This was true for all congeners except BDE-28 ($p = 0.33$), that had no contribution to the ΣBFR levels in any of the bird species tested. The post hoc Tukey test results from the one-way ANOVA indicated that the occurrence of various congeners differed statistically significantly between species ($p < 0.05$). These significant differences were as follows:

- BDE-47: Cattle Egret had lower levels than granivores
- BDE-99: The African Darter had higher levels than the African Sacred Ibis and Crowned Lapwing. The Red-knobbed Coot and granivores had higher levels than the African Darter and the granivores had higher levels than the White-breasted Cormorant and Heron *spp* as well.
- BDE-100: The African Darter had higher levels than the Cattle Egret, Red-knobbed Coot and Heron *spp*, while the White-breasted Cormorant had higher levels than the Cattle Egret and Granivores.
- BDE-153: The African Sacred Ibis differed from all species except the White-breasted Cormorant and Heron *spp*. Heron *spp*. differed from Cattle Egrets and the Red-knobbed Coots.
- BDE-154: The African Sacred Ibis had higher levels than the Cattle Egret, Red-knobbed Coot and granivores, while the African Darter had higher levels than all species except the African Sacred Ibis and White-breasted Cormorant. The White-breasted Cormorant had higher levels than the Cattle Egret, Crowned-Lapwing, Red-knobbed Coot and granivores.
- BDE-183: The African Sacred Ibis had higher levels than all other species. Heron *spp* had higher levels than all species except the Crowned Lapwing and the granivores.
- BDE-206: The African Sacred Ibis had higher levels than the Cattle Egret, African Darter and granivore.
- BDE-207, -209: The African Sacred Ibis had higher levels than all other species
- BDE-208: The African Sacred Ibis had higher levels than all other species, while the African darter higher levels than the granivores.
- HBCD: The African Sacred Ibis had higher levels than all other species except the White-breasted Cormorant.

For the African Sacred Ibis, the dominant PBDEs were BDE-209 (36%) and -183 (28%). HBCD contributed only 6% to the ΣBFR. In the African Darter the dominant BFRs were BDE-154 (30%), -HBCD (21%), and -100 (15%). For the White-breasted Cormorant the predominant PBDEs were BDE-154, -153, and -47 and for Heron *sp* the dominant BFRs

were BDE-183 (42%), -153 (33%), and -207 (6%). HBCD contributed 14% to the ΣBFR in White-breasted cormorants and did not contribute to the ΣBFR in Heron *sp* tested.

In the Cattle Egret the dominant congers were BDE -99 (85%) and in the Crowned Lapwing BDE -47 (31%), -99 (22%), and -153 (12%). HBCD did not contribute to the ΣBFR in either species. In the eggs of the Red-knobbed Coot the dominant congeners were BDE -99 (66%) and -47 (20%), with HBCD contributing 5% to the total. For the granivore species the Cape Sparrow, the dominant congener was BDE-99 (91%) followed by HBCD contributing 3% to the total. In eggs of the Southern Masked Weaver, the dominant congeners were BDE -47 (46%), -99 (43%) and HBCD (3%). In the single pooled sample for the Cape Turtle Dove the dominant congener was BDE-99 (95%).

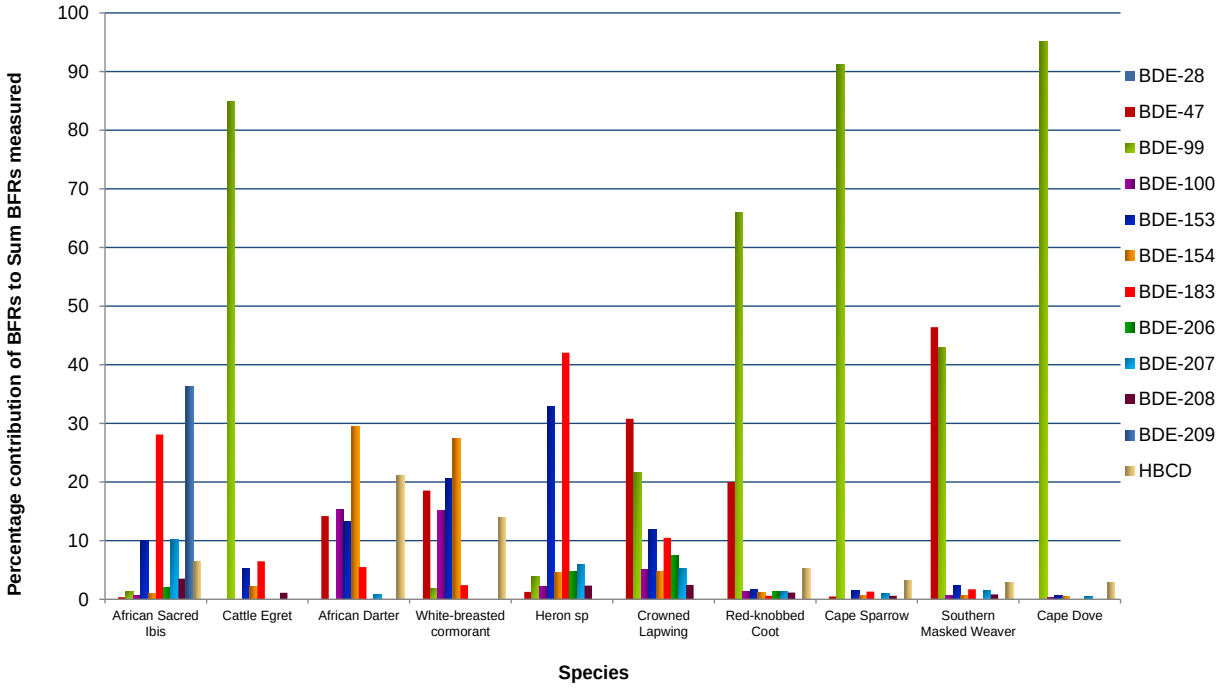


Figure 18: Relative contributions of median concentrations of PBDE congeners and HBCD to ΣBFR residues in eggs (congeners below LOD were assigned a zero value)

4.1.4 PCA analysis of BFRs found in South African wild bird eggs

A PCA analysis was conducted to investigate the congener profiles of BFRs in the wild-bird eggs, as well as the possible effect of geographical distribution. In the PCA-biplot, (Figure 19) factor 1 explained 37% and factor 2, 32% (Tables S2 and S3) of the variance in the dataset.

Factor 1 was a contrast between BDE-207, -208, and -183 against BDE-47, -100, -28, and -154. Factor 2 was mainly a contrast between BDE-99 and BDE-153.

Figure 19: Biplot of the PCA for PBDEs and HBCD for all wild bird species. The African Sacred Ibis, the only scavenging species, is indicated in orange, piscivores in blue, insectivores/omnivores in black and granivores in green. Data labels for individual bird eggs used as listed in Tables 14-18

Bird species were distributed along both factor 1 and factor 2 on the PCA-biplot. Factor 1 on the PCA-biplot clustered together the African Sacred Ibis and herons that were associated with BDE-207, -208, -183 and the African Darter, White-breasted Cormorant and a single Southern Masked Weaver that was associated with BDE-47, -100, -28, and -154.

Factor 2 clustered together the granivores and insectivores/omnivores with BDE-99 and the piscivores and African Sacred Ibis with BDE-154. HBCD, BDE-206 and -209 did not seem to contribute significantly to either factor 1 or 2, indicating a low impact on the variance within the dataset. In the PCA biplot, the wild bird species did not cluster according to the various geographical sampling areas as discussed in Chapter 3.

4.1.5 Investigating the effect of trophic guild on the occurrence of BFRs in eggs

As discussed previously (section 2.5), the level of organic pollutants are influenced by the trophic level of a specific organism. The effect of trophic guild on the concentration of individual PBDE congers as well as the total BFRs were investigated using a one-way ANOVA and subsequently the post hoc Tukey test.

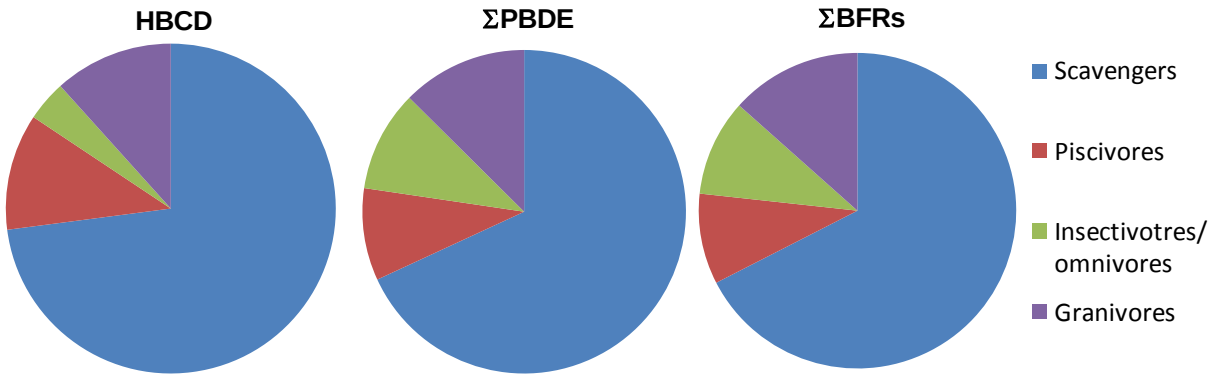


Figure 20: The contribution of the various trophic guilds to HBCD, ΣPBDEs, and ΣBFRs.

There is a statistically significant difference in the concentration of ΣBFRs between the various trophic guilds (one way ANOVA; $p = 0.009$). The concentration of ΣBFRs within the various trophic guilds in dedreasing order were (Figure 20): scavengers (46 ng g^{-1} wm) > granivores (9.1 ng g^{-1} wm) > insectivores/omnivores (6.7 ng g^{-1} wm) > (6.3 ng g^{-1} wm). The pattern for ΣPBDEs remained the same: scavengers (43 ng g^{-1} wm) > granivores (8 ng g^{-1} wm) > insectivores/ omnivores (6.4 ng g^{-1} wm) > piscivores (5.9 ng g^{-1} wm) (Figure 20). However, the pattern for HBCD differed from the above mentioned: scavengers (2.8 ng g^{-1} wm) > granivores (0.44 ng g^{-1} wm) ≥ piscivores (0.43 ng g^{-1} wm) > insectivores/ omnivores (0.15 ng g^{-1} wm)

(Figure 20). The post hoc Tukey test indicated that scavengers had a significantly higher level of ΣPBDE than piscivores ($p = 0.01$), and their ΣBFRs were higher than both piscivores and insectivores/omnivores ($p = 0.04$ and $p = 0.01$ respectively). However, levels of ΣBFRs in scavengers did not differ significantly from granivores ($p = 0.75$) (Figure 20).

Furthermore, the one-way ANOVA analysis indicated that all BFR congeners with the exception of BDE-28 and -209 differed significantly between the various trophic guilds ($p \leq 0.05$) (Figure 21). Levels of BDE-47 and -99 were statistically significantly higher in granivores than in other guilds (post hoc Tuckey test: $p = 0.01$ and $p < 0.0001$ respectively; Figure 20). Scavengers had significantly higher levels of BDE- 183, -207, -208 and HBDC ($p \leq 0.05$; Figure 21) while levels of BDE-206 in scavengers were also significantly higher than those in piscivores and granivores ($p = 0.02$ and 0.01 respectively). The insectivores/omnivores differed statistically significantly from piscivores for BDE-100 ($p = 0.03$) and BDE-154 ($p = 0.023$) and for BDE-153 from scavengers ($p = 0.01$).

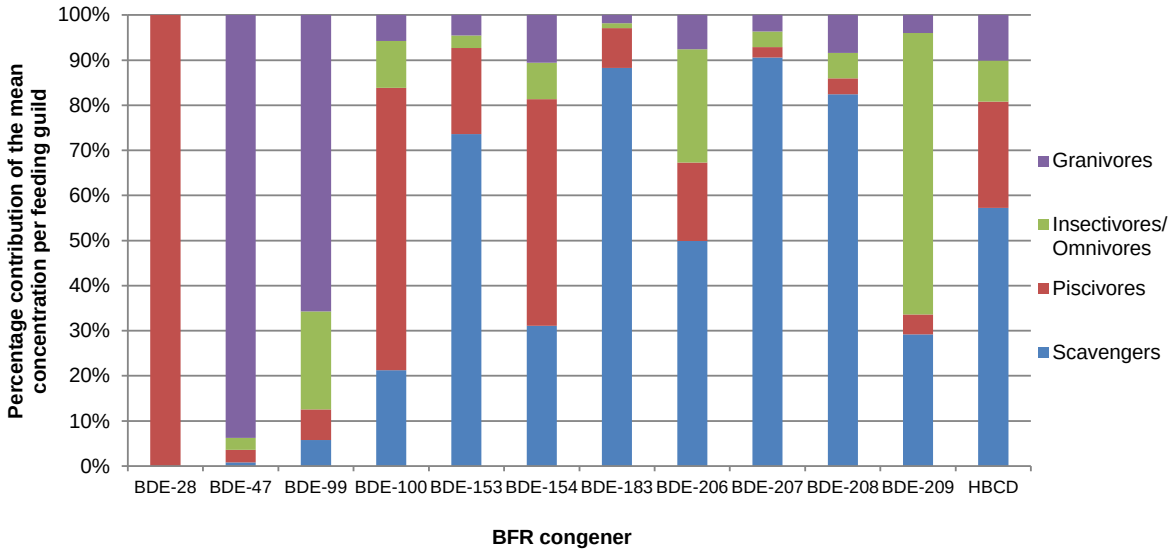


Figure 21: The percentage distribution of congeners between eggs from various feeding guilds.

4.1.6 Feeding habitat and congener profiles of BFRs in wild bird eggs

Although the trophic level of an organism is important in the exposure profiles of organohalogens, other factors such as feeding habitat have to be considered (Lavoie *et al*., 2010). To investigate the possible effect of habitat on concentration and profiles of BFRs, a one-way ANOVA and post hoc Tukey analysis were preformed using log-transformed data. The variation in concentrations of the BFRs between the different feeding habitats is shown in Figure 22.

Most of the species investigated can be distinctly grouped into aquatic and terrestrial habitats, with the exception of the African Sacred Ibis. As discussed in section 3.1.4, the African Sacred Ibis often frequents aquatic ecosystems feeding on any available prey. However, the African Sacred Ibis adapted to feed on organic matter from waste dumps, sewage works and can feed on ploughed fields (Bouwman *et al*., 2008). Therefore the African Sacred Ibis was placed into a feeding habitat class of its own and is labelled "combined" in Figure 22. All other birds were placed in to the terrestrial (Crowned Lapwing, Cattle Egret, Southern Masked Weaver, Cape Sparrow, and Cape Turtle Dove) or aquatic habitat (White-breasted Cormorant, African Darter, Black-crowned Night Heron, Grey Heron, Black-headed Heron, and Red-knobbed Coot). The feeding habitat preference of the wild birds has a statistically significant impact on the concentration of BFRs ($p = 0.00$). This was clear for all BDE congeners except BDE-28 and -47 ($p = 0.29$ and $p = 0.74$, respectively) (Figure 22).

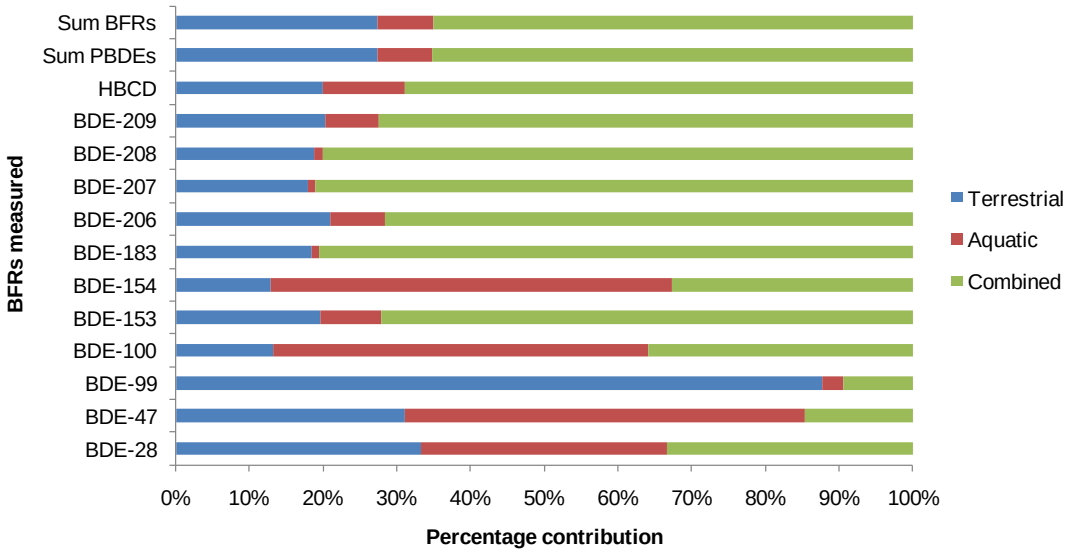


Figure 22: Variation in BFRs between the different feeding habitats.

When studying the post hoc Tukey results it was clear that the majority of the difference was attributed to the combined habitat (African Sacred Ibis) that had significantly higher levels than both the aquatic and terrestrial habitats for BDE-153, -183, -206, -207, -208, and -209 as well as HBCD, ΣPBDEs, and ΣBFRs. This pattern changed for BDE-99, as it had higher levels in terrestrial birds ($p < 0.0001$). For BDE-100 and -154, the terrestrial habitat had statistically significantly higher levels than those from both the combined and aquatic habitat ($p < 0.004$ and $p < 0.004$).

4.1.7 BFR-congeners and eggshell thickness

The correlations between the various PBDEs to HBCD, ΣPBDE and ΣBFRs were investigated using the product-moment correlation test on log-transformed data. To be regarded as a statistically significant correlation, the following conditions had to be met: $p < 0.05$ and $r > \pm 0.6$, where r represents the correlation coefficient. Although a correlation does not need to be linear to be statistically significant ($p < 0.05$) for the purpose of this analysis only correlation coefficients (r) greater than ±0.6 were considered.

The correlation matrix indicates that BDE-153,-183 and -207 were positively correlated to HBCD ($p < 0.05$ and $r > 0.6$). ΣPBDEs were correlated to BDE-183, -207, -208, and -209 ($p < 0.05$ and $r > 0.6$).

When looking at eggshell thickness, no correlations were meaningful except for granivores, where BDE-100 and -208 showed a significant negative correlation to eggshell thickness ($p = 0.05$; $r = -0.7$ for both, Figure 23). Due to the small sample size, the analysis was repeated using the Spearman Rank order correlation for the granivores, using this correlation matrix. BDE-100, 153, 183, and -207, showed statistically significant negative correlations with eggshell thickness ($p < 0.05$; $r > 0.6$).

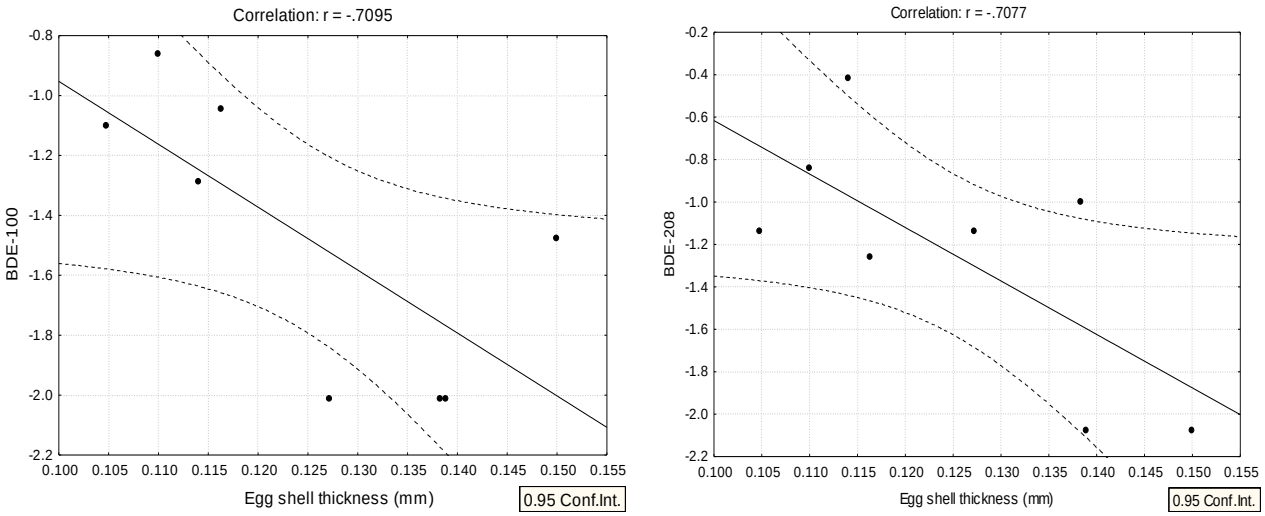


Figure 23: Scatter plot of BDE-100 and -208 against eggshell thickness for granivores (n = 7), indicating significant negative correlation ($p < 0.05$; $r > 0.6$)

4.2 OCPs IN WILD BIRD EGGS

4.2.1 Levels and congener profiles of OCPs

All wild bird eggs sampled had measurable levels of OCPs, with HCB, β-HCH, oxychlordane, *cis*-nonachlor, *p,p'*-DDT *p,p'*-DDE and *p,p'*-DDD at levels above the LOD. The compounds with the lowest detection frequency were mirex, found in 72% of samples, and *trans*-chlordane, found in 77%. *Cis*-chlordane, *trans*-nonachlor, α-HCH, and γ-HCH were found in greater than 90% of eggs analysed. Descriptive statics for the levels of OCPs in the various bird species is summarised in Table 21, and levels found in individual bird eggs are given in Table S4 (supplementary data). Median ΣOCPs ranged between 4.2 – 620 ng g^{-1} wm.

The mean concentration of OCPs differed statistically significantly between the different bird species (one-way ANOVA, $p < 0.0001$). Only the mean concentrations of α-HCH, *trans*-chlordane, *p,p'*-DDT, and mirex did not differ significantly between species (one-way ANOVA, $p > 0.05$). The highest concentration of OCPs was measured in the White-breasted Cormorant and the lowest level in the Cape Turtle Dove. An African Darter egg collected in Kempton Park had the highest level of OCPs found in this study (2 600 ng g^{-1} wm) with the lowest concentration of OCPs measured in a Cape Sparrow egg collected in Sasolburg (4 ng g^{-1} wm).

ΣDDT contributed greater than 70% to the ΣOCPs found in all species, except for the Crowned Lapwing and Cape Sparrow (Figure 24). For the Crowned Lapwing, chlordanes contributed 80% of the ΣOCP measured, while ΣDDT contributed only 4%. In the case of the Cape Sparrow, ΣDDT contributed 58% to the ΣOCPs, followed by the ΣHCH that contributed 22%. HCB contributed between 0.2 - 22% to the ΣOCPs. The highest concentration of HCB was measured in the Crowned Lapwing (19 ng g^{-1} wm) and this concentration was statistically significantly higher than all other bird species (one-way ANOVA; $p < 0.05$). Mirex contributed less than 1% to the total OCPs with the exception of the Cattle Egret that had 5% (Figure 24). ΣHCH contribution was relatively constant ranging from 1% – 5%, except for the Cape Sparrow where the contribution of ΣHCH was 22% (Figure 24).

Of DDT and its metabolites, the most abundant compound in all species was *p,p'*-DDE. *p,p'*-DDE contributed between 60 – 90% of ΣDDTs, followed by *p,p'*-DDT (1 – 26%) and *p,p'*-DDD (1 – 7%) (Figure 25). The median concentration of *p,p'*-DDE ranged between 2.5 – 600 ng g^{-1} wm. The levels in bird species in declining order was White-breasted Cormorant > African Darter > Heron > Red-knobbed Coot > African Sacred Ibis > Southern Masked Weaver > Cattle Egret > Crowned Lapwing > Cape Turtle Dove > Cape Sparrow.

Table 21: OCP concentrations (ng g^{-1} wm) in eggs of wild bird species

Species	Min	Max	Mean	Median	SD	Species	Min	Max	Mean	Median	SD
Piscivorous species: *African Darter (n = 13)*						**Insectivores/Omnivores: *Cattle Egret (n = 6)***					
%Lipid	1.32	6.45	4.56	5.41	1.65	%Lipid	5.81	8.59	6.89	6.52	1.01
HCB	0.69	8.57	3.40	2.52	2.51	HCB	0.29	1.76	0.66	0.46	0.56
α-HCH	0.03	10.59	1.01	0.10	2.90	α-HCH	ND	0.03	0.02	0.02	0.01
β-HCH	1.19	138.66	31.16	9.28	48.86	β-HCH	0.04	1.29	0.40	0.14	0.50
γ-HCH	0.06	9.48	2.49	2.17	2.85	γ-HCH	ND	0.04	0.02	0.02	0.02
ΣHCH	*1.28*	*155.94*	*34.66*	*13.34*	*52.18*	*ΣHCH*	*0.11*	*1.33*	*0.44*	*0.17*	*0.49*
oxychlordane	0.63	25.81	5.83	3.02	6.89	oxychlordane	0.03	0.71	0.26	0.10	0.28
trans-chlordane	ND	0.61	0.13	0.05	0.17	*trans*-chlordane	ND	0.02	0.01	0.01	0.01
cis-chlordane	0.02	3.63	0.52	0.20	0.96	*cis*-chlordane	ND	0.04	0.02	0.01	0.01
trans-nonachlor	0.04	8.87	0.96	0.28	2.39	*trans*-nonachlor	0.02	0.88	0.28	0.08	0.36
cis-nonachlor	0.11	3.66	1.13	0.73	1.14	*cis*-nonachlor	0.03	0.14	0.06	0.04	0.05
ΣChlordanes	*1.09*	*42.59*	*8.56*	*4.34*	*10.98*	*ΣChlordanes*	*0.11*	*1.57*	*0.63*	*0.23*	*0.68*
p,p'-DDE	122.11	2441.62	523.33	292.69	625.70	*p,p'*-DDE	2.72	51.64	17.79	8.02	19.63
p,p'-DDD	0.05	6.82	1.44	0.52	2.01	*p,p'*-DDD	0.02	0.09	0.04	0.03	0.03
p,p'-DDT	0.48	146.11	12.89	1.40	40.06	*p,p'*-DDT	0.14	1.86	0.58	0.30	0.66
ΣDDT	*122.83*	*2591.95*	*537.66*	*294.39*	*664.32*	*ΣDDT*	*2.92*	*53.59*	*18.41*	*8.35*	*20.29*
Mirex	ND	1.34	0.63	0.79	0.41	Mirex	0.22	1.74	0.64	0.47	0.57
ΣOCPs	*140.71*	*2648.14*	*584.91*	*339.00*	*687.30*	*ΣOCPs*	*4.14*	*57.16*	*20.78*	*10.68*	*20.93*
White-breasted Cormorant (n = 4)						***Crowned Lapwing (n = 6)***					
%Lipid	3.80	5.41	4.22	3.83	0.79	%Lipid	8.71	15.51	11.48	11.12	2.21
HCB	0.79	2.48	1.57	1.50	0.73	HCB	4.81	79.12	24.58	19.33	27.65
α-HCH	0.09	0.22	0.15	0.16	0.05	α-HCH	0.01	0.08	0.04	0.03	0.03
β-HCH	7.50	15.88	11.57	11.45	3.64	β-HCH	0.31	7.34	2.33	1.11	2.67
γ-HCH	2.92	6.37	4.20	3.76	1.59	γ-HCH	0.07	1.26	0.48	0.43	0.42
ΣHCH	*10.51*	*19.56*	*15.93*	*16.82*	*4.27*	*ΣHCH*	*0.40*	*7.90*	*2.85*	*2.05*	*2.74*
oxychlordane	3.47	8.68	5.62	5.16	2.52	oxychlordane	1.18	124.71	37.28	19.17	46.16
trans-chlordane	ND	0.11	0.06	0.05	0.04	*trans*-chlordane	0.02	0.71	0.32	0.30	0.28
cis-chlordane	0.12	0.21	0.15	0.15	0.04	*cis*-chlordane	0.07	3.99	1.63	1.37	1.44
trans-nonachlor	0.27	0.42	0.32	0.29	0.07	*trans*-nonachlor	2.96	463.91	156.22	83.53	176.16
cis-nonachlor	0.36	0.81	0.57	0.56	0.19	*cis*-nonachlor	0.35	55.80	17.42	8.38	21.06
ΣChlordanes	*4.45*	*10.23*	*6.72*	*6.09*	*2.71*	*ΣChlordanes*	*4.58*	*647.46*	*212.87*	*112.74*	*243.88*
p,p'-DDE	422.02	921.55	634.60	597.42	232.36	*p,p'*-DDE	0.98	25.03	6.84	3.52	9.14
p,p'-DDD	0.30	0.50	0.36	0.32	0.09	*p,p'*-DDD	0.21	0.71	0.40	0.37	0.18
p,p'-DDT	0.37	0.70	0.49	0.45	0.15	*p,p'*-DDT	0.44	2.64	1.42	1.38	0.86
ΣDDT	*422.88*	*922.24*	*635.45*	*598.34*	*232.38*	*ΣDDT*	*1.80*	*28.38*	*8.66*	*5.27*	*10.00*
Mirex	0.11	0.36	0.23	0.23	0.11	Mirex	0.46	6.30	2.30	0.98	2.44
ΣOCPs	*446.88*	*947.53*	*659.90*	*622.59*	*235.99*	*ΣOCPs*	*12.04*	*672.90*	*251.26*	*183.50*	*243.11*
Heron spp (n = 6)						***Red-knobbed Coot (n = 6)***					
%Lipid	5.60	7.26	6.25	6.18	0.62	%Lipid	12.43	14.11	13.28	13.26	0.54
HCB	0.20	3.68	1.86	1.76	1.66	HCB	0.28	2.17	0.85	0.51	0.75
α-HCH	ND	0.02	0.01	0.01	0.01	α-HCH	0.01	0.04	0.03	0.03	0.01
β-HCH	0.45	9.54	3.73	3.43	3.45	β-HCH	0.37	2.26	1.66	1.74	0.67
γ-HCH	0.03	0.35	0.10	0.03	0.13	γ-HCH	0.02	0.32	0.12	0.09	0.12
ΣHCH	*0.57*	*9.57*	*3.83*	*3.63*	*3.40*	*ΣHCH*	*0.40*	*2.38*	*1.81*	*1.97*	*0.74*
oxychlordane	0.55	8.75	3.45	1.18	3.99	oxychlordane	0.04	0.54	0.37	0.39	0.18
trans-chlordane	ND	0.05	0.02	0.01	0.02	*trans*-chlordane	0.02	0.08	0.04	0.04	0.02
cis-chlordane	0.04	0.59	0.22	0.08	0.25	*cis*-chlordane	0.04	0.37	0.20	0.18	0.14
trans-nonachlor	0.36	6.94	2.61	1.18	2.91	*trans*-nonachlor	ND	0.26	0.13	0.09	0.10
cis-nonachlor	0.04	0.56	0.24	0.12	0.22	*cis*-nonachlor	0.04	0.75	0.29	0.23	0.24
ΣChlordanes	*1.07*	*16.38*	*6.53*	*2.43*	*7.34*	*ΣChlordanes*	*0.17*	*1.72*	*1.03*	*1.08*	*0.56*
p,p'-DDE	45.64	272.98	151.33	158.59	81.86	*p,p'*-DDE	4.38	74.99	37.16	34.04	33.56
p,p'-DDD	0.05	15.94	4.62	0.38	6.98	*p,p'*-DDD	ND	0.09	0.05	0.05	0.03
p,p'-DDT	0.12	14.22	3.47	0.93	5.49	*p,p'*-DDT	0.13	0.64	0.34	0.32	0.17
ΣDDT	*46.96*	*287.70*	*159.42*	*166.79*	*87.46*	*ΣDDT*	*4.60*	*75.70*	*37.55*	*34.41*	*33.69*
Mirex	0.11	37.74	7.17	0.80	15.03	Mirex	ND	ND	ND	ND	ND
ΣOCPs	*74.23*	*318.48*	*178.82*	*190.00*	*91.43*	*ΣOCPs*	*6.47*	*81.23*	*41.24*	*38.25*	*34.83*

Table 21 continued: OCP concentrations (ng g^{-1} wm) in eggs of various wild bird species

Species	Min	Max	Mean	Median	SD	Species	Min	Max	Mean	Median	SD
Scavenger species : African *Sacred Ibis (n = 16)*						**Granivores**	***Cape Turtle Dove (n = 3: 1 pool of 3 eggs)***				
%Lipid	4.12	7.60	5.83	5.61	0.91	%Lipid	8.52				
HCB	0.37	2.56	1.10	0.91	0.62	HCB	0.24				
α-HCH	ND	0.02	0.01	0.01	0.01	α-HCH	0.01				
β-HCH	0.33	3.45	1.22	1.11	0.70	β-HCH	0.06				
γ-HCH	0.07	1.65	0.60	0.40	0.53	γ-HCH	0.11				
Σ*HCH*	*0.41*	*5.09*	*1.84*	*1.58*	*1.12*	Σ*HCH*	*0.19*				
oxychlordane	0.51	3.07	1.63	1.53	0.71	oxychlordane	0.20				
trans-chlordane	ND	2.38	0.18	0.03	0.59	trans-chlordane	0.05				
cis-chlordane	0.03	0.21	0.09	0.08	0.04	cis-chlordane	0.04				
trans-nonachlor	0.44	4.64	1.88	1.42	1.16	trans-nonachlor	0.35				
cis-nonachlor	0.03	0.29	0.12	0.12	0.07	cis-nonachlor	0.04				
Σ*Chlordanes*	*1.06*	*8.00*	*3.90*	*3.46*	*2.05*	Σ*Chlordanes*	*0.67*				
p,p'-DDE	3.87	90.64	39.62	33.40	21.31	*p,p'*-DDE	2.88				
p,p'-DDD	0.14	2.88	0.56	0.24	0.88	*p,p'*-DDD	0.04				
p,p'-DDT	1.17	26.95	8.55	7.20	6.46	*p,p'*-DDT	0.16				
Σ*DDT*	*5.18*	*120.47*	*48.74*	*42.75*	*27.58*	Σ*DDT*	*3.08*				
Mirex	ND	0.18	0.11	0.11	0.04	Mirex	ND				
Σ*OCPs*	*7.06*	*127.20*	*55.68*	*48.89*	*28.45*	Σ*OCPs*	*4.18*				
Granivores: Cape Sparrow (n = 9: 3 pools of 3 eggs)						***Southern Masked Weaver (n = 8: 4 pools of 2 eggs)***					
%Lipid	3.85	7.14	5.84	6.54	1.75	%Lipid	2.97	6.06	4.46	4.40	1.29
HCB	0.63	2.56	1.41	1.05	1.02	HCB	0.50	2.11	0.97	0.63	0.77
α-HCH	0.02	0.03	0.03	0.03	0.00	α-HCH	0.01	0.03	0.02	0.02	0.01
β-HCH	0.05	0.62	0.27	0.13	0.31	β-HCH	0.12	1.84	0.76	0.54	0.76
γ-HCH	0.19	0.45	0.33	0.35	0.13	γ-HCH	0.06	8.47	2.64	1.02	3.92
Σ*HCH*	0.26	1.11	0.63	0.51	0.44	Σ*HCH*	*0.19*	*10.33*	*3.43*	*1.59*	*4.68*
oxychlordane	0.08	0.24	0.15	0.12	0.08	oxychlordane	0.21	1.89	1.05	1.04	0.81
trans-chlordane	0.05	0.06	0.06	0.06	0.00	trans-chlordane	0.03	0.08	0.06	0.07	0.02
cis-chlordane	0.04	0.08	0.06	0.05	0.02	cis-chlordane	0.06	0.54	0.21	0.12	0.22
trans-nonachlor	0.07	0.17	0.13	0.15	0.05	trans-nonachlor	0.15	0.46	0.31	0.31	0.12
cis-nonachlor	0.04	0.09	0.05	0.04	0.03	cis-nonachlor	0.08	0.32	0.21	0.23	0.10
Σ*Chlordanes*	0.31	0.61	0.44	0.40	0.15	Σ*Chlordanes*	*0.58*	*3.13*	*1.84*	*1.82*	*1.14*
p,p'-DDE	0.55	8.96	4.01	2.53	4.40	*p,p'*-DDE	2.75	42.02	19.60	16.82	18.73
p,p'-DDD	0.02	0.09	0.05	0.06	0.03	*p,p'*-DDD	0.09	0.62	0.37	0.39	0.28
p,p'-DDT	0.07	0.23	0.14	0.12	0.08	*p,p'*-DDT	0.06	0.61	0.22	0.10	0.27
Σ*DDT*	0.80	9.08	4.21	2.74	4.33	Σ*DDT*	*2.93*	*42.70*	*20.19*	*17.57*	*19.07*
Mirex	ND	ND	ND	ND	ND	Mirex	ND	0.28	0.28	ND	_
Σ*OCPs*	4.02	11.44	6.69	4.62	4.12	Σ*OCPs*	*6.28*	*58.28*	*26.50*	*20.71*	*24.52*

ND not detected

For *p,p'*-DDE, the mean concentration of the African Darter and White-breasted Cormorant were significantly higher than all other species (one-way ANOVA; $p < 0.05$) with the exception of the Heron *spp*. and between each other (one-way ANOVA; $p > 0.05$). The median concentration of *p,p'*-DDT ranged between 0.1 – 7.2 ng g^{-1} wm, with the highest concentrations measured in the African Sacred Ibis. However, there was no statistically significant difference among the species (one-way ANOVA; $p > 0.05$).

For *p,p'*-DDD, concentrations were between 0.04 and 0.52 ng g^{-1} wm, with the highest levels measured in the African Darter. The concentration of *p,p'*-DDD was significantly higher in Heron *spp*. compared to the Cattle Egret, Crowned Lapwing, Red-knobbed Coot, African

Sacred Ibis and granivore species (one-way ANOVA; $p < 0.05$). The mean ΣDDT concentration in the African Darter was significantly higher than in the African Sacred Ibis, Cattle Egret, Crowned Lapwing, Red-knobbed Coot and Granivores ($p < 0.05$). The ΣDDT was significantly higher in the White-breasted Cormorant compared to the African Sacred Ibis and granivores ($p < 0.05$).

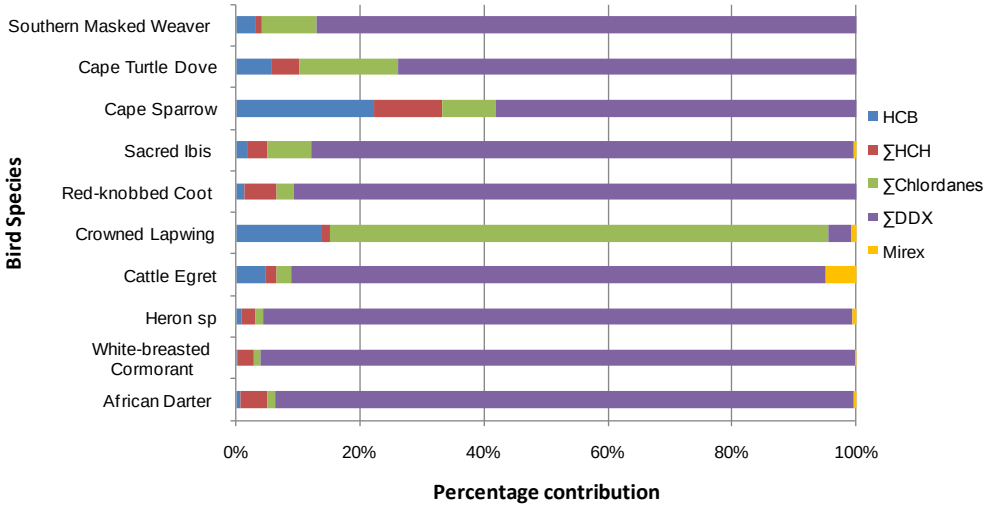


Figure 24: The percentage contribution of the various OCP groups the total OCPs in the eggs of various bird species studied

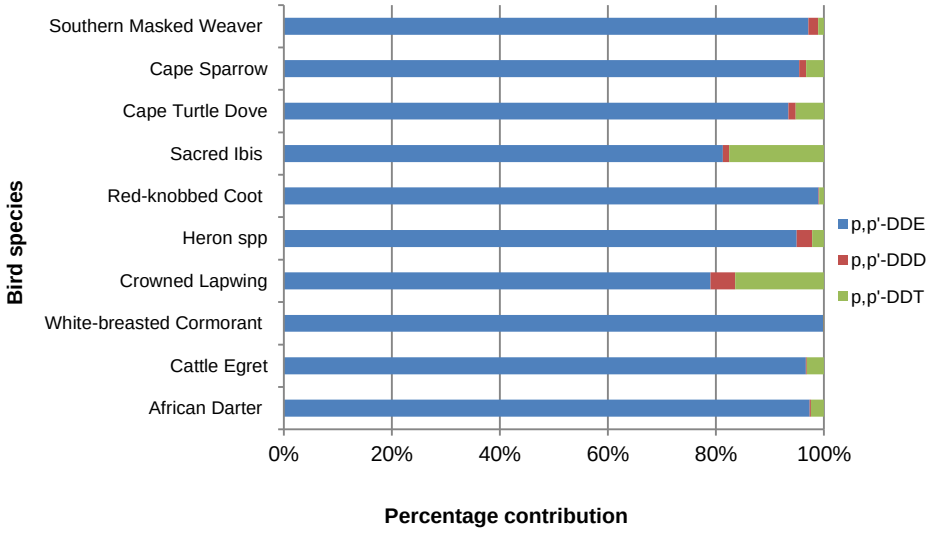


Figure 25: The percentage contribution of DDT and its metabolites to the ΣDDT found in the eggs of various bird species studied

The predominant HCH-isomer was β-HCH that contributed between 26 – 99% to ΣHCH, followed by γ-HCH (1 - 68%) and α-HCH (0.2 - 7%; Figure 26). The median concentration of β-HCH was between 0.13 – 11 ng g^{-1} wm, with the levels decreasing in bird species as follows: White-breasted Cormorant > African Darter > Heron > Red-knobbed Coot > African Sacred Ibis = Crowned Lapwing > Southern Masked Weaver > Cape Sparrow > Cape Turtle Dove. The median concentration of γ-HCH ranged between 0.02 - 3.8 ng g^{-1} wm, whereas the median concentration of α-HCH ranged between 0.01 - 0.16 ng g^{-1} wm. The highest levels for both γ- and α-HCH were measured in the White-breasted Cormorant. For β-HCH and ΣHCH the African Darter had statistically significantly higher levels than the African Sacred Ibis (one-way ANOVA, $p = 0.01$) and in the case of γ-HCH the African Sacred Ibis, Cattle Egret, and Heron *spp*. had statistically significantly lower levels than the White-breasted Cormorant ($p < 0.05$).

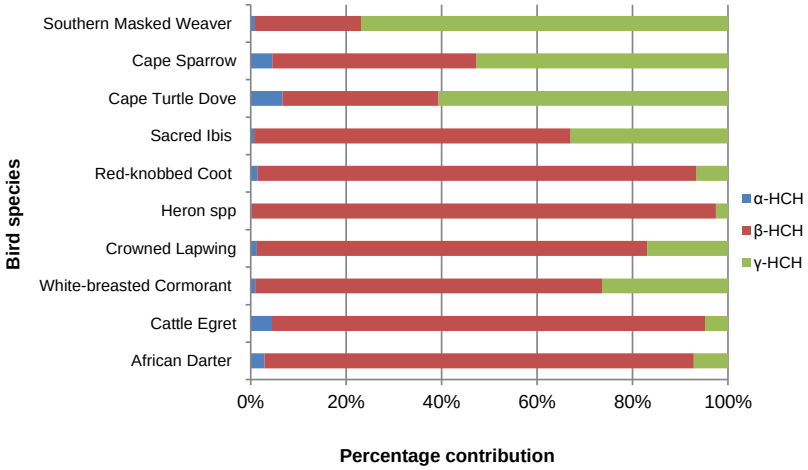


Figure 26: The percentage contribution of the HCH isomers to the ΣHCH found in eggs of various species studied

In the case of chlordanes, no single congener was as prominent as *p,p'*-DDE or β-HCH for the OCPs. In general, the largest contribution to Σchlordanes was made by oxychlordane (17 - 83%) and *trans*-nonachlor (6 – 74%), followed by *cis*-nonachlor (5 – 24%), *cis*-chlordane (1 – 19%) and *trans*-chlordane (0.2 – 14%) (Figure 27 and Table 21). Median levels for oxychlordane ranged between 0.12 – 19 ng g^{-1} wm, with concentrations in descending order: Crowned Lapwing > White-breasted Cormorant > African Darter > African Sacred Ibis > Heron *spp*. > Southern Masked Weaver > Red-knobbed Coot > Cape Turtle Dove > Cape Sparrow > Cattle Egret. For *trans*-nonachlor concentrations were between 0.08 and 86 ng g^{-1} wm, with concentrations in species in declining order: Crowned Lapwing > African Sacred Ibis > Heron *sp* > Cape Turtle Dove > Southern Masked Weaver > White-breasted Cormorant > African Darter >

Cape Sparrow > Cattle Egret> Red-knobbed Coot. The pattern of the various chlordane constituents ranged between species (Figure 27). For the White-breasted Cormorant and the African Darter, the pattern was oxychlordane > *cis*-nonachlor > *trans*-nonachlor > *cis*-chlordane > *trans*-chlordane, while the pattern for the Crowned Lapwing and Heron *sp* was *trans*-nonachlor > oxychlordane > *cis*-nonachlor > *cis*-chlordane > *trans*-chlordane. The African Sacred Ibis and Southern Masked Weaver showed the pattern of oxychlordane > *trans*-nonachlordane > *cis*-nonachlor > *cis*-chlordane > *trans*-chlordane, while the pattern for the Cape Turtle Dove and Cape Sparrows was *trans*-nonachlor > oxychlordane > *trans*-chlordane > *cis*-chlordane > *cis*-nonachlor. The Cattle Egret and Red-knobbed Coot had unique patterns from all other species. For the Cattle Egret it was oxychlordane > *trans*-nonachlor > *cis*-nonachlor > *trans*-chlordane > *cis*-chlordane and for the Red-knobbed Coot it was oxychlordane > *cis*-nonachlor > *cis*-chlordane > *trans*-nonachlor > *trans*-chlordane. The concentrations of oxychlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor and Σchlrodanes were statistically significantly lower (one-way ANOVA; $p < 0.05$) in the Cattle Egret compared to all other species.

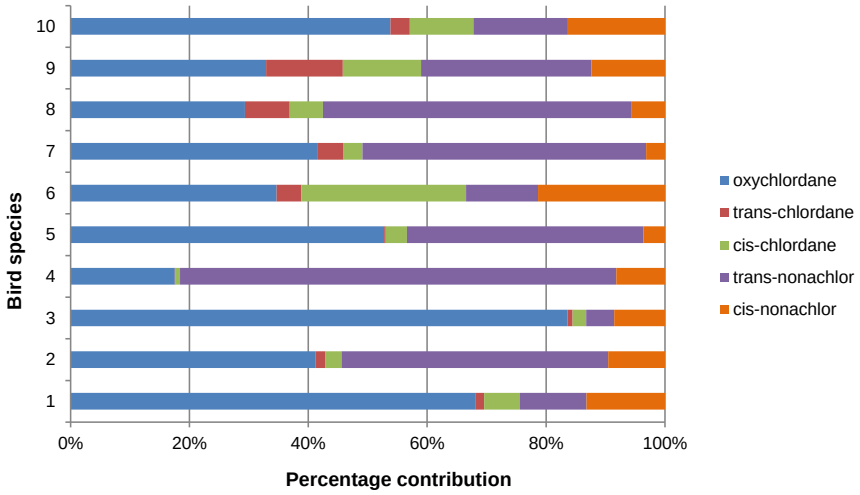


Figure 27: The percentage contribution of the various chlordane components and metabolite oxychlordane to the Σchlordanes found in the eggs of various bird species studied

4.2.2 PCA analysis of OCPs found in South African wild bird eggs

A PCA analysis was performed to assess the congener profile and geographical distribution of OCPs in wild bird eggs. In the PCA-biplot (Figure 28), factor 1 explained 33% of the variance in the data, followed by factor 2 with 19%, factor 3 with 14% and factor 4 with 10%. Only factors 1 and 2 will be discussed. However, the full factor scores and factor loadings tables are given in the supplementary data (Table S5 and S6).

Factor 1 was mainly a contrast between *p,p'*-DDE, β-HCH, and *trans*- and *cis*-nonachlor. Cattle Egrets and the piscivore species separated along factor 1 in the PCA-biplot. The Crowned Lapwings were associated with the *trans*-and *cis*-nonachlor, while the piscivore species were associated with *p,p'*-DDE and β-HCH. Factor 2 was a contrast between α-HCH, γ-HCH and DDT and *p,p'*-DDD. The granivores, African Darter, African Sacred Ibis and Heron *sp* were separated along factor 2 on the PCA-biplot. The Southern Masked Weaver, Cape Sparrow, and the African Darter were associated with α-HCH and γ-HCH, whereas the African Sacred Ibis and Heron *sp* were associated with DDT and DDE. As with BFRs, there was no clear clustering according to geographical distribution of the collected eggs.

4.2.3. Investigating the effect of trophic guild on the occurrence of OCPs in eggs

To assess the relationship between trophic guilds with the concentration of OCPs found in wild bird eggs, a one-way ANOVA analysis was performed. This analysis indicated a statistically significant difference in the means of the various trophic guilds ($p < 0.0001$). The concentration of ΣOCPs (490 ng g^{-1} wm) was statistically higher in piscivores ($p < 0.05$) compared to all other species, followed by insectivores/omnivores (100 ng g^{-1} wm) and scavengers (49 ng g^{-1} wm) (Figure 28). The lowest concentration of ΣOCPs was measured in the granivores (7.2 ng g^{-1} wm) (Figure 29).

For HCB, the highest concentrations were measured in insectivores/omnivores (8.7 ng g^{-1} wm) followed by piscivores (2.4 ng g^{-1} wm) (Figure 29). The concentration of HCB was similar in the scavengers (0.19 ng g^{-1} wm) and granivores (0.63 ng g^{-1} wm) (Figure 28). The difference between species was, however, not statistically significant ($p > 0.05$).

The mean concentration of ΣHCH was highest in piscivores (24 ng g^{-1} wm) (Figure 29), while the median concentration of scavengers (1.6 ng g^{-1} wm), insectivores/omnivores (1.4 ng g^{-1} wm), and granivores (0.75 ng g^{-1} wm) were similar (Figure 29). The differences between the trophic guilds were not statistically significant ($p > 0.05$).

The concentration of Σchlordanes between the various guilds had a unique pattern. The highest concentration of Σchlordanes was measured in piscivores (4.4 ng g^{-1} wm), closely followed by scavengers (3.5 ng g^{-1} wm) and insectivores/omnivores (1.4 ng g^{-1} wm). This should not be confused with the individual species data where the Crowned Lapwing had the highest level of chlordanes. The lowest concentrations were measured in the granivores (0.64 ng g^{-1} wm). However, the differences between the various guilds were not statistically significant ($p > 0.05$).

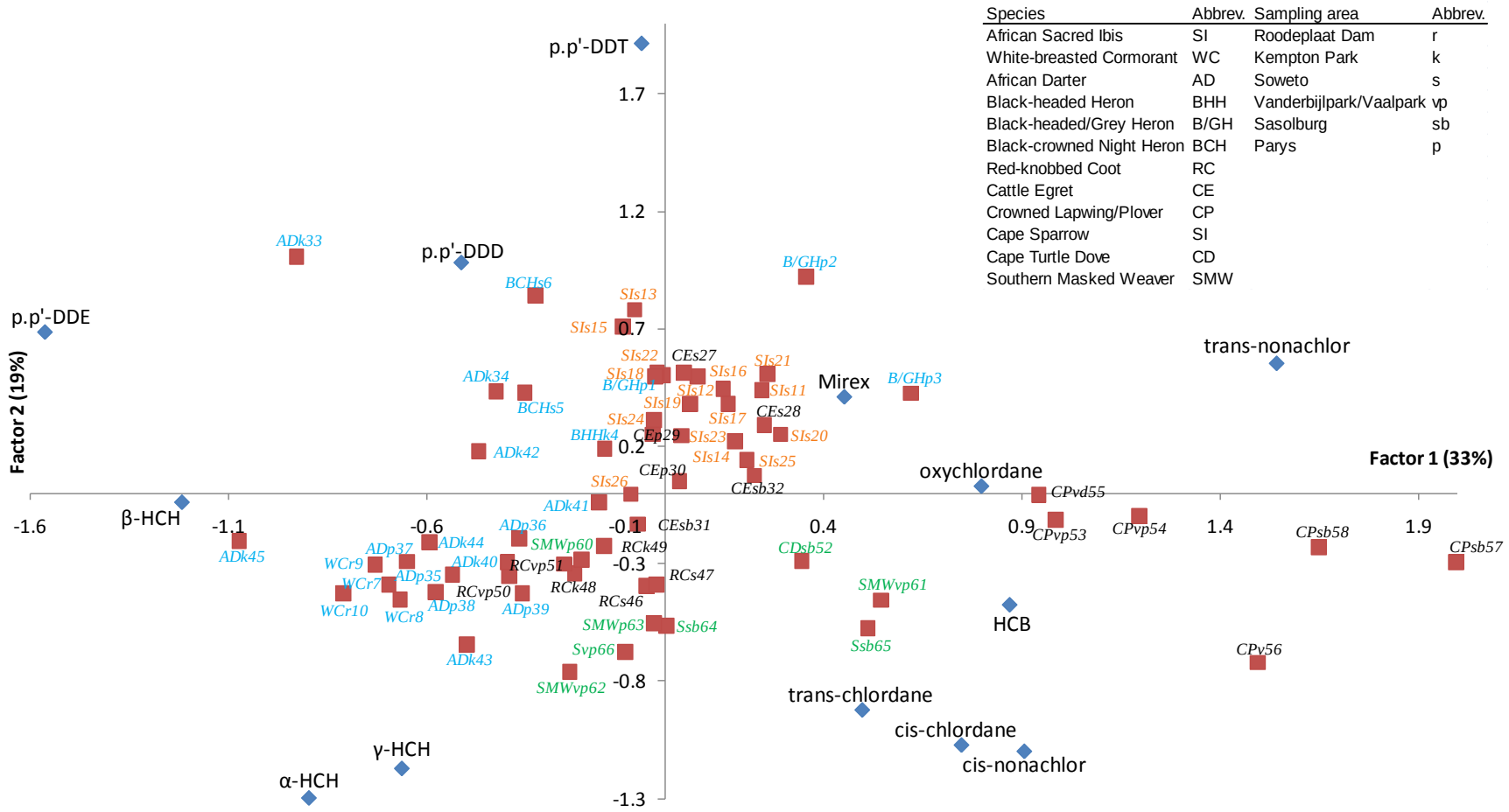


Figure 28: Biplot of the PCA for OCPs for all wild bird species. The African Sacred Ibis, the only scavenging species, is indicated in orange, piscivores in blue, insectivores/omnivores in black and granivores in green. Data labels for individual bird eggs used as listed in Tables 14-18

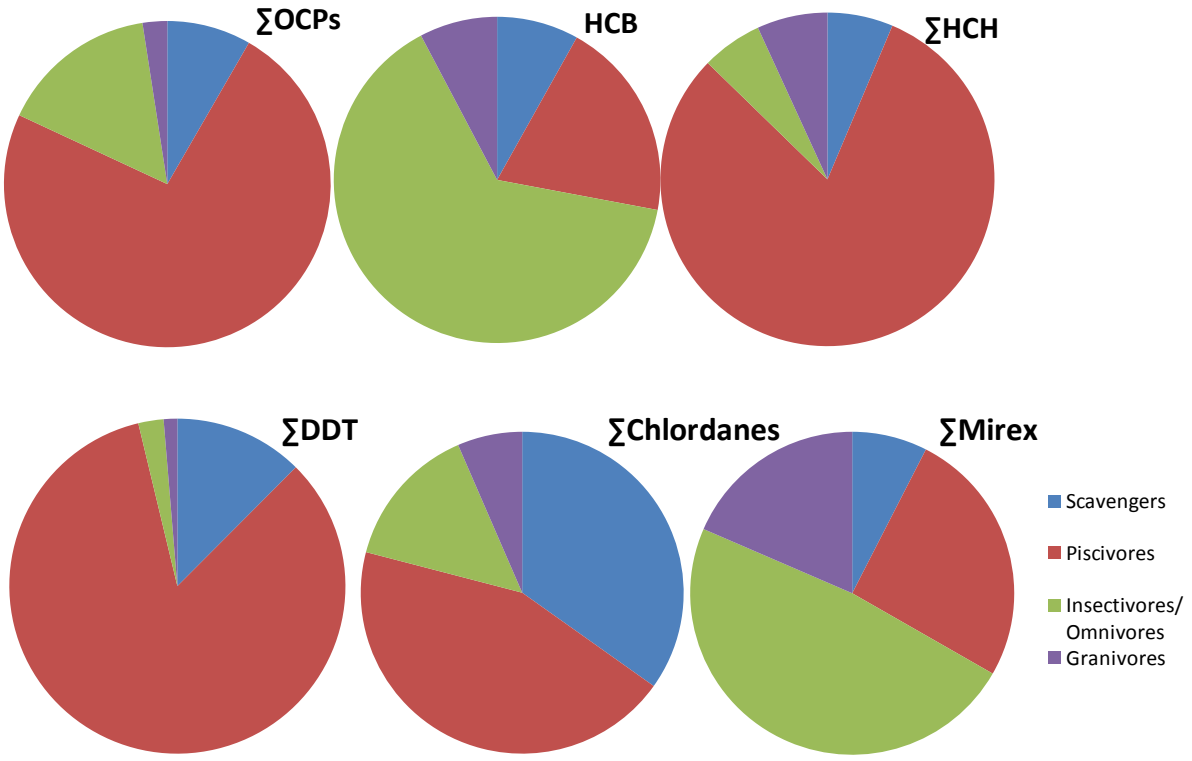


Figure 29: The contribution of the various trophic guilds to ΣOCPs (Scavengers, Piscivores Insectivores/omnivores Granivores)

Additionally, the patterns of the individual OCP-groups between the trophic guilds were analysed using log transformed data in a one-way ANOVA with a post hoc Tukey test. All compounds differed significantly between various feeding guilds with the exception of HCB ($p = 0.17$) and the chlordanes: *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor and Σchlordanes ($p > 0.05$). As discussed previously, DDTs were the most prominent pollutant and mirex only occurred at low to ND levels (Figure 30). Mirex differed significantly between scavengers and piscivores ($p < 0.0001$), where piscivores had higher levels than scavengers did. Mirex concentrations in granivores were significantly lower than concentrations in both insectivores/omnivores and piscivores ($p = 0.00$, respectively). The occurrence of ΣHCH differed in piscivores from all other guilds ($p < 0.05$), while ΣDDT differed between all species except for the granivores and insectivores/omnivores that did not differ statistically significantly from one another ($p = 0.47$).

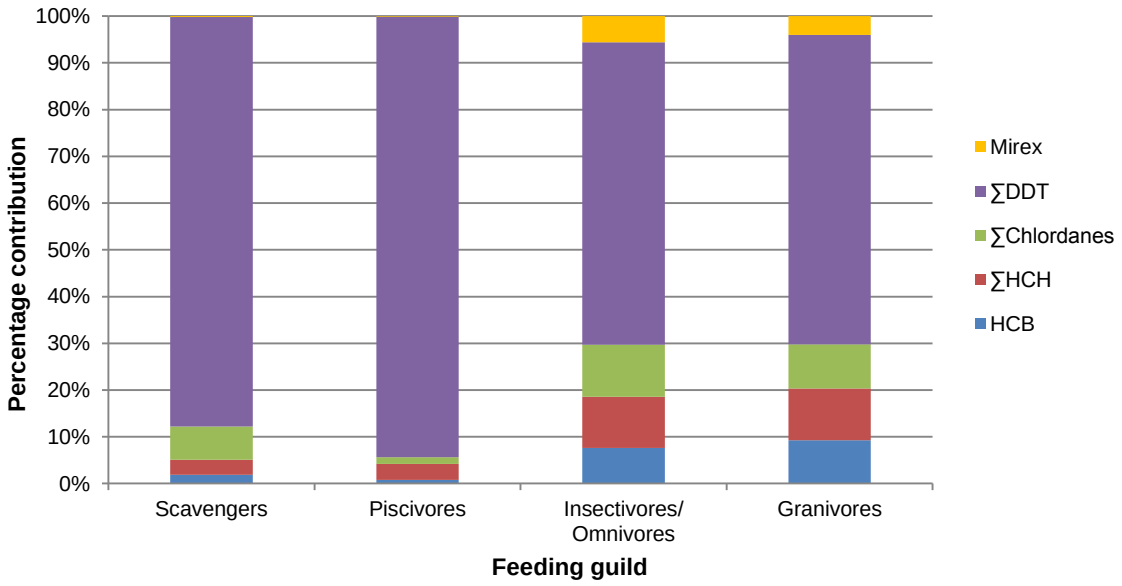


Figure 30: The percentage distribution of OCPs between eggs from various feeding guilds

4.2.4 Feeding habitat and congener profiles of OCPs in wild bird eggs

The influence of habitat on the occurrence of OCPs was investigated through a One-Way ANOVA and post hoc Tukey test with log-transformed data. Birds feeding in different habitats had significantly different OCP patterns ($p = 0.00$). This was true for all compounds except HCB, γ-HCH, oxychlordane, *cis*-chlordane, *trans*-chlordane, Σchlordanes, and mirex ($p < 0.05$) (Figure 31).

The eggs of birds feeding in aquatic habitats had significantly higher levels than eggs of birds feeding in the combined and terrestrial habitats ($p < 0.05$) for α-HCH, β-HCH, ΣHCH, and ΣOCPs (Figure 31). Eggs of birds feeding in the aquatic and combined habitats differed from one another for both *trans*- and *cis*-nonachlor (both, $p = 0.03$) while eggs of birds feeding in aquatic and terrestrial habitats differed for *p,p'*-DDD ($p = 0.04$). All the habitat type bird eggs differed significantly from one another ($p < 0.05$) for *p,p'*-DDE, *p,p'*-DDT and ΣDDT residue levels (Figure 31). However, bird eggs from combined habitat users (African Sacred Ibis) had statistically significantly higher levels of *p,p'*-DDT, and piscivores eggs had statistically significantly higher levels of *p,p'*-DDE, *p,p'*-DDD and ΣDDT residues.

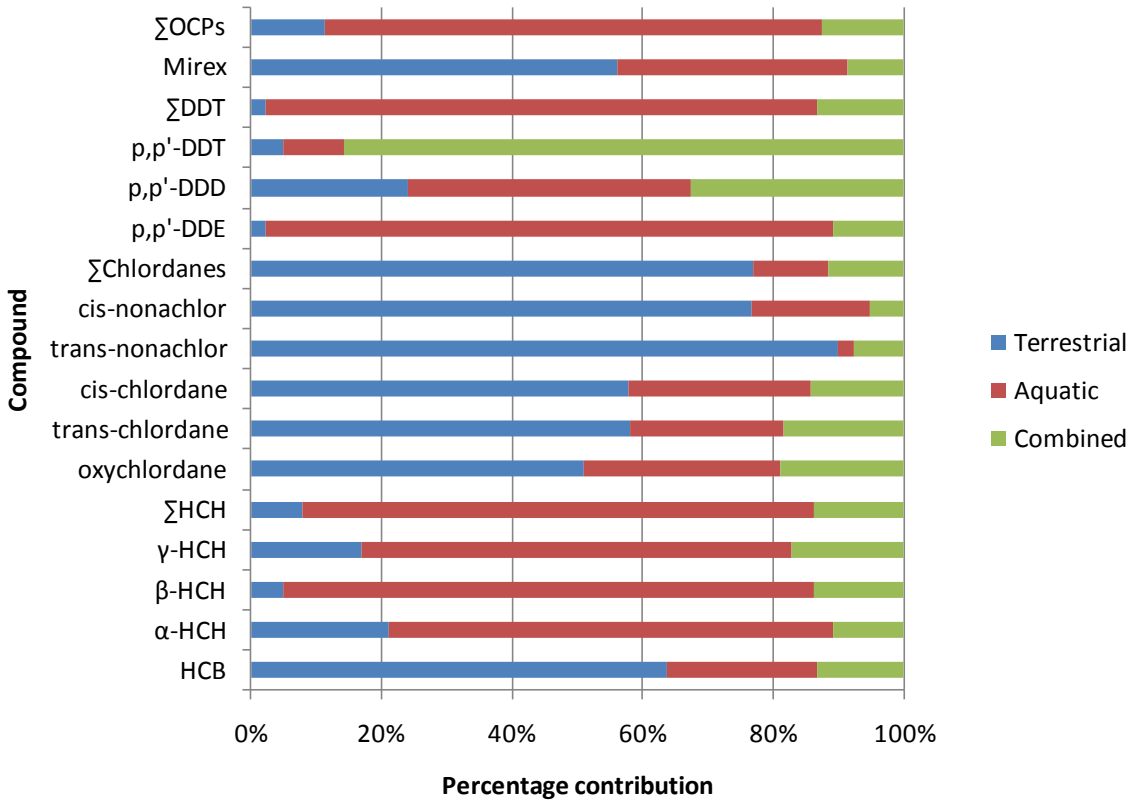


Figure 31: Variation in OCPs between the different feeding habitats

4.2.5 OCPs and egg parameters

To investigate the relationship between the various OCP and between the OCPs and egg parameters, a product-moment correlation on log-transformed data was performed as described in section 4.1.7. The results of the various OCPs are presented in Figures 32 and 33.

ΣOCPs were significantly correlated ($p < 0.05$; $r > \pm 0.6$) to β-HCH, oxychlordane, *cis*-chlordane, *cis*-nonachlor, *p,p'*-DDE, and *p,p'*-DDD. Mirex, *p,p'*-DDT and γ-HCH were the only compounds not significantly correlated to any other OCP. ΣChlordanes were correlated to HCB, ΣHCH and all of the other chlordane-compounds with the exception of *trans*-chlordane. *Trans*-chlordane was only correlated to *cis*-chlordane whereas *cis*-chlordane was additionally correlated to HCB, ΣHCH and oxychlordane. The sum of the individual OCP classes correlations were not discussed in relation to each other or the ΣOCPs since the autocorrelation effect could have impacted correlations significantly.

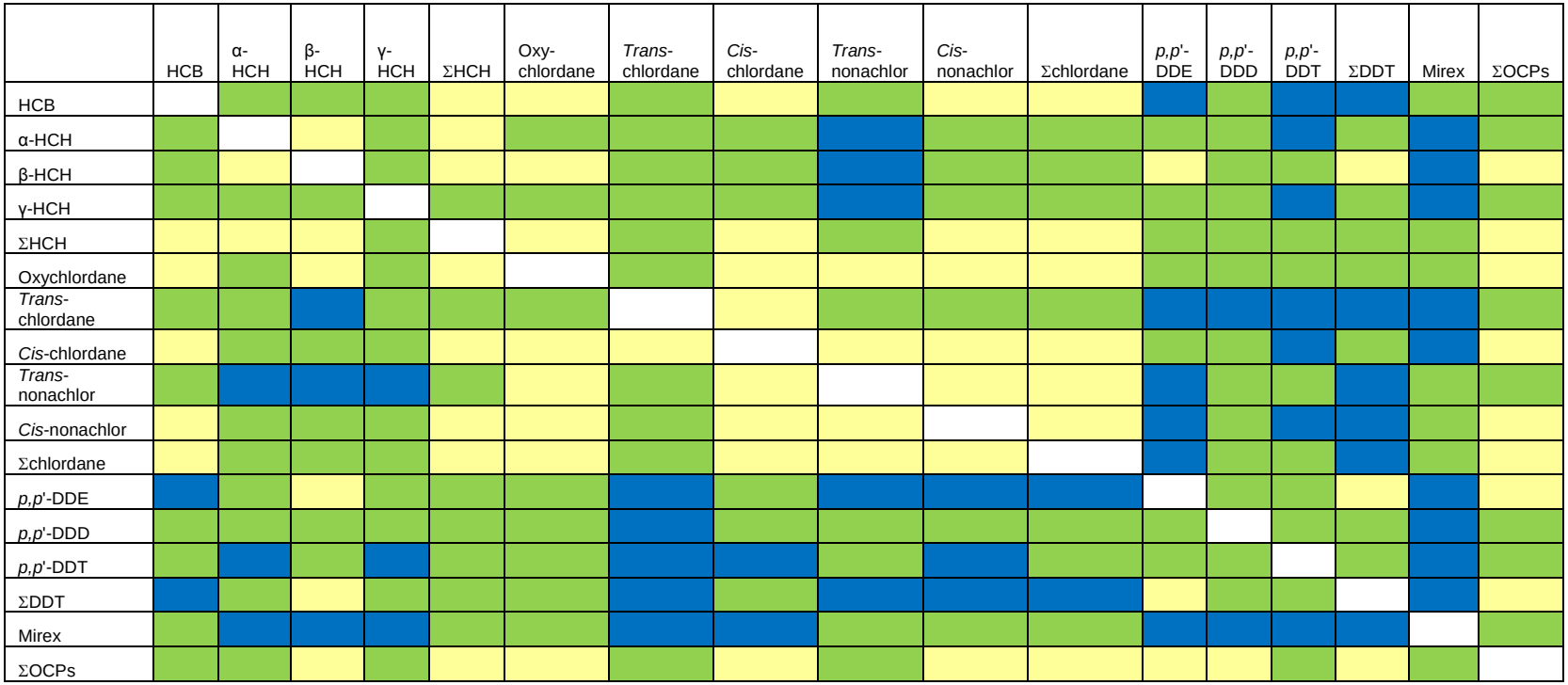


Figure 32: Correlation matrix for individual OCP congeners, blue indicates no significant correlation ($p > 0.05$), green a significant correlation ($p < 0.05$) with $r < 0.6$, and yellow a statistically significant correlation ($p < 0.05$; $r > 0.6$)

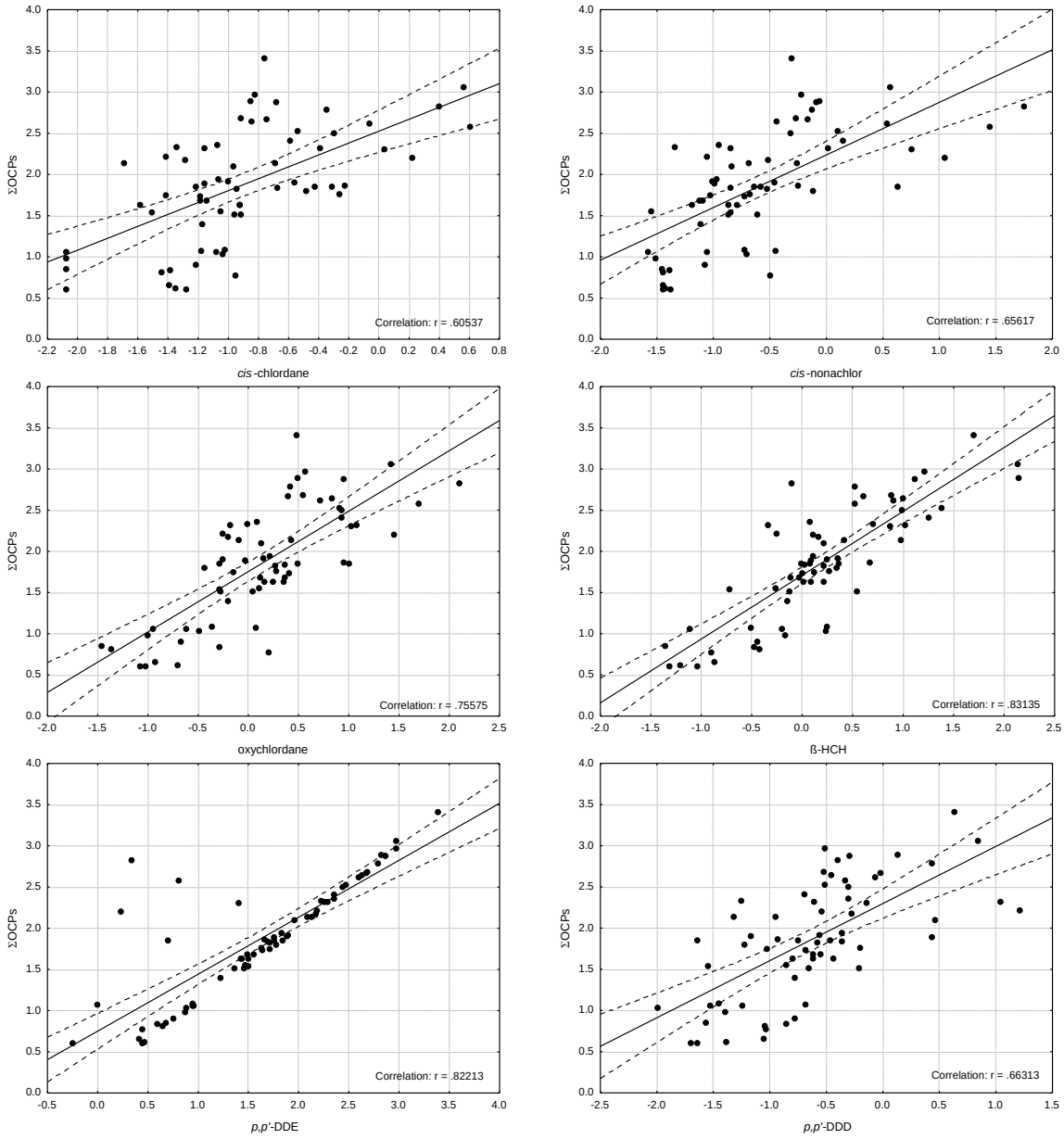


Figure 33: Statistically significant correlations between ΣOCPs and individual compounds, with 95% confidence intervals for ΣOCPs

Oxychlordane was correlated with HCB and β-HCH, as well as the nonachlors. *Cis*-nonachlor was additionally correlated with HCB, ΣHCH, and *trans*-nonachlor. *Trans*-nonachlor was correlated with the same suite of compounds as *cis*-nonachlor, with the exception of the HCH-isomers and ΣOCPs. The correlation of the HCH isomers between one another was only significant for α-HCH and β-HCH. Other than mentioned above, HCB was also correlated to ΣHCH. When looking at the correlation between eggshell thickness and OCPs, in all species,

there were no linear correlations ($r > \pm 0.6$). However, there were positive statistically significant correlations ($p < 0.05$) for *p,p'*-DDE, *p,p'*-DDT and ΣOCPs. The relationship between eggshell thickness and OCPs were further analysed on a species-specific basis.

For the African Sacred Ibis, Cattle Egret, African Darter, Crowned Lapwing and Red-knobbed Coot, there was no statistically significant linear correlation between eggshell thickness and OCPs. However, in granivores there was a significant negative correlation between *p,p'*-DDD and eggshell thickness ($p = 0.02$; $r = -0.78$; Figure 34). This was confirmed using the Spearman Rank order correlation ($p < 0.05$; $r = -0.88$).

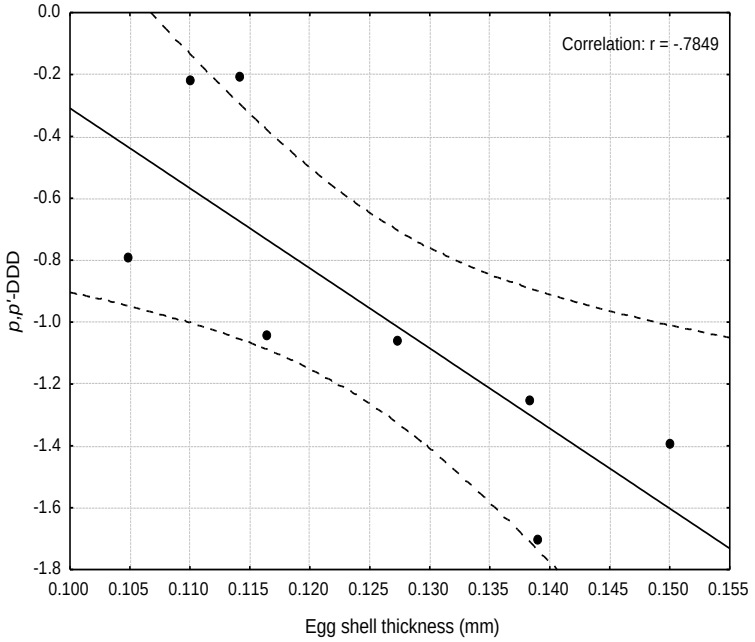


Figure 34: Correlation between eggshell thickness and *p,p'*-DDD in granivores, showing the 95% confidence intervals

4.3 PCBs IN WILD BIRD EGGS

PCBs were quantified in all wild bird eggs analysed. Ten PCB congeners (CB-52, -101, -105, -138, -153, -167, -180, -187, -194, -196, and 206) were present in all samples. Of the 34 congeners analysed, 20 (CB-28, -31, -47, -66, -74, -99, -110, -114, -118, -123, -128, -137, -141, -149, -156, -157, -170, -183, -189, and -199) were present in greater than 80% of samples. CB-151 and -209 were present in greater than 70% of samples, whereas CB-56, having the lowest occurrence, was measured in only 19% of samples.

The most dominant congeners in all species that had a mean contribution of greater than 10%, were CB-138, -153, and -180 (Table 22). Median ΣPCBs values ranged between 0.92 – 300 ng g^{-1} wm (Table 22). The White-breasted Cormorant had the highest concentration, where CB-153 contributed 27% to ΣPCBs (Table 22) followed by the African Darter (250 ng g^{-1} wm) with CB-153 contributing 28%. On an individual sample basis, the highest concentration of ΣPCBs was measured in an African Darter (840 ng g^{-1} wm) and the lowest in the Cape Turtle Dove (0.68 ng g^{-1} wm) (Supplementary data: Table S7).

To investigate the distribution of PCB congeners between various species as well as to assess the possible relationship with geographically distribution, a PCA analysis was performed. In the PCA-biplot (Figure 35), factor 1 explained 32% of the variance in the dataset and was mainly a contrast between: CB-170, -180, -156, -183, -157, -153, and-138 with negative scores, and CB-52, -47, -101, -110, and -56 with positive scores. Bird species grouped along factor 1 in the PCA-biplot were the heron *spp*., White-breasted, Cormorants and African Darter with negative loadings and the Cape Turtle Dove, Cattle Egret and Red-knobbed Coot with positive loadings.

Factor 2 explained 22% of the variance in the data and was a contrast between CB-194, -196, -209, -206, -199, and -189 with negative scores against CB-28, -74, -105, -66, -99, -31, and -123 with positive scores. Bird species were not strongly separated along factor 2 in the PCA-biplot. However, the Southern Masked Weavers and Red-knobbed Coots with positive loadings were separated from the Heron *spp* with negative loadings on the second axes of the PCA-bipolot. Factor 3 and 4 explained 11 and 7% of the variance in the data set respectively. Neither of the factors will be discussed further. However, full factor loading and factor scores are given in the supplementary data (Table S8 and S9).

Table 22: PCB concentrations (ng g^{-1} wm) in eggs of various wild bird species

Species	Min	Max	Mean	Median	SD	Species	Min	Max	Mean	Median	SD
Piscivorous species: *African Darter (n = 13)*						Piscivorous species: *White-breasted Cormorant (n = 4)*					
%Lipid	1.32	6.45	4.56	5.41	1.65	%Lipid	3.80	5.41	4.22	3.83	0.79
CB28	0.24	34.53	5.74	2.41	9.12	CB28	4.12	8.60	6.59	6.83	2.04
CB31	ND	3.82	0.82	0.44	1.10	CB31	0.57	1.74	1.12	1.09	0.48
CB47	ND	0.56	0.22	0.21	0.18	CB47	0.06	0.12	0.08	0.08	0.03
CB52	0.14	1.15	0.32	0.23	0.28	CB52	0.44	0.93	0.63	0.58	0.23
CB56	ND	0.24	0.05	0.02	0.06	CB56	0.02	0.05	0.04	0.03	0.01
CB66	0.36	18.30	3.87	2.05	4.79	CB66	5.56	10.64	8.46	8.83	2.33
CB74	0.75	20.15	6.38	5.32	5.06	CB74	7.75	17.16	11.96	11.47	4.16
CB99	1.65	9.84	4.71	4.07	2.48	CB99	6.67	13.04	10.08	10.30	2.97
CB101	0.29	2.80	0.73	0.45	0.68	CB101	0.65	0.97	0.83	0.85	0.13
CB105	0.89	12.72	6.14	5.97	3.32	CB105	0.22	0.37	8.96	8.98	2.76
CB110	0.14	5.11	0.97	0.36	1.40	CB110	5.78	12.08	0.29	0.29	0.06
CB114	0.07	1.22	0.64	0.62	0.35	CB114	0.55	1.10	0.81	0.79	0.24
CB118	3.61	40.10	21.58	19.34	11.01	CB118	14.80	31.43	22.82	22.51	7.35
CB123	0.06	1.30	0.56	0.48	0.33	CB123	0.44	0.85	0.63	0.62	0.18
CB128	0.62	7.91	3.28	2.57	2.25	CB128	1.52	3.12	2.27	2.22	0.72
CB137	0.09	1.09	0.48	0.39	0.34	CB137	0.48	0.87	0.68	0.70	0.17
CB138	6.35	120.68	39.61	27.22	34.88	CB138	30.80	65.31	47.83	47.61	15.60
CB141	0.08	1.63	0.35	0.22	0.43	CB141	0.10	0.19	0.15	0.15	0.04
CB149	0.12	5.09	1.03	0.59	1.38	CB149	0.33	0.60	0.47	0.47	0.11
CB151	0.08	3.58	0.66	0.43	0.94	CB151	0.03	0.10	0.06	0.06	0.03
CB153	17.30	276.42	94.65	69.40	74.48	CB153	57.66	124.37	85.67	80.32	29.86
CB156	1.18	11.28	6.18	6.53	3.26	CB156	2.66	5.61	3.88	3.62	1.33
CB157	0.31	2.96	1.66	1.52	0.86	CB157	0.80	1.82	1.23	1.15	0.45
CB167	0.96	6.40	3.47	3.17	1.77	CB167	1.74	3.24	2.42	2.35	0.63
CB170	3.62	49.13	19.89	13.58	15.23	CB170	9.31	21.55	14.58	13.73	5.50
CB180	8.78	117.75	47.59	32.72	35.10	CB180	20.86	45.65	33.29	33.33	11.26
CB183	1.12	15.65	6.39	5.23	4.34	CB183	2.56	5.88	4.29	4.35	1.51
CB187	1.71	38.98	12.02	8.74	10.56	CB187	5.54	15.28	11.05	11.68	4.10
CB189	0.10	1.13	0.54	0.52	0.31	CB189	0.19	0.40	0.26	0.23	0.10
CB194	2.19	15.33	8.45	8.25	4.00	CB194	5.42	8.70	6.54	6.01	1.54
CB196	1.12	16.16	6.53	7.03	3.93	CB196	2.51	6.72	4.19	3.75	1.87
CB199	0.11	0.87	0.48	0.38	0.26	CB199	0.18	0.42	0.29	0.29	0.11
CB206	1.29	11.17	6.50	6.11	2.77	CB206	4.89	13.13	7.70	6.39	3.75
CB209	0.21	1.56	0.64	0.52	0.39	CB209	0.63	1.36	0.85	0.71	0.34
∑CBs	58.13	835.91	313.11	248.90	223.38	∑CBs	195.94	415.16	300.99	296.44	98.03
Piscivorous species: *Heron sp (n = 6)*						Scavenger species: African S*acred Ibis (n = 16)*					
%Lipid	5.60	7.26	6.25	6.18	0.62	%Lipid	4.12	7.60	5.83	5.61	0.91
CB28	0.09	1.85	0.53	0.32	0.67	CB28	0.13	1.56	0.59	0.48	0.37
CB31	0.04	0.47	0.18	0.07	0.20	CB31	0.03	0.34	0.13	0.11	0.07
CB47	0.00	0.06	0.03	0.03	0.01	CB47	0.09	0.58	0.24	0.23	0.11
CB52	0.03	0.11	0.08	0.08	0.03	CB52	0.11	0.31	0.16	0.16	0.05
CB56	ND	ND	0.01	0.01	0.00	CB56	ND	ND	0.01	0.01	0.00
CB66	0.09	1.69	0.54	0.34	0.61	CB66	0.13	1.76	0.71	0.62	0.37
CB74	0.16	2.53	0.82	0.41	0.93	CB74	0.14	1.87	0.84	0.73	0.46
CB99	0.18	1.50	0.81	0.74	0.60	CB99	0.24	3.18	1.07	0.89	0.66
CB101	0.07	0.09	0.08	0.08	0.01	CB101	0.07	0.77	0.24	0.21	0.16
CB105	0.20	1.88	0.89	0.62	0.81	CB105	0.19	2.98	1.23	1.10	0.71
CB110	ND	0.08	0.04	0.02	0.03	CB110	0.05	1.24	0.26	0.16	0.28
CB114	0.02	0.18	0.08	0.06	0.06	CB114	0.01	0.22	0.10	0.08	0.06
CB118	0.82	5.34	2.91	2.81	2.20	CB118	0.57	7.16	3.18	2.77	1.72
CB123	0.02	0.09	0.05	0.04	0.03	CB123	0.02	0.29	0.13	0.12	0.07
CB128	0.04	0.44	0.27	0.29	0.17	CB128	0.17	2.11	0.77	0.62	0.47
CB137	ND	0.15	0.09	0.09	0.06	CB137	0.03	0.39	0.18	0.15	0.09
CB138	0.16	9.74	4.89	4.43	3.58	CB138	1.94	10.41	6.43	6.69	2.51
CB141	0.01	0.06	0.03	0.03	0.02	CB141	0.03	0.27	0.15	0.15	0.07
CB149	0.03	0.36	0.15	0.07	0.15	CB149	0.13	1.10	0.57	0.55	0.24
CB151	ND	0.14	0.05	0.01	0.06	CB151	0.03	0.26	0.15	0.14	0.08
CB153	5.17	51.53	23.52	14.17	19.70	CB153	3.58	14.87	9.98	10.30	3.53
CB156	0.47	3.58	1.78	1.37	1.33	CB156	0.29	2.35	1.21	1.19	0.52
CB157	0.12	0.69	0.35	0.25	0.24	CB157	0.05	0.53	0.24	0.22	0.11
CB167	0.31	1.55	0.94	0.89	0.48	CB167	0.12	0.75	0.43	0.41	0.17
CB170	0.82	7.24	3.41	2.42	2.63	CB170	1.22	6.12	3.61	3.95	1.42
CB180	2.61	31.57	12.80	7.30	11.21	CB180	3.46	17.33	9.28	9.58	3.75
CB183	0.41	2.75	1.46	1.18	1.00	CB183	0.61	2.74	1.62	1.71	0.66
CB187	0.37	3.17	1.84	1.95	1.13	CB187	0.91	5.23	3.04	3.11	1.20
CB189	0.06	0.52	0.28	0.22	0.19	CB189	0.05	0.25	0.15	0.16	0.05
CB194	2.45	11.66	7.61	7.55	3.77	CB194	1.28	6.58	3.28	3.46	1.47
CB196	0.69	4.26	3.01	3.61	1.50	CB196	1.41	5.58	3.11	3.15	1.20
CB199	0.02	0.25	0.09	0.05	0.09	CB199	0.05	0.16	0.11	0.12	0.04
CB206	3.54	21.48	11.67	10.16	8.22	CB206	2.09	10.46	4.86	4.16	2.46
CB209	0.49	2.83	1.43	1.24	0.84	CB209	0.23	1.49	0.67	0.60	0.36
∑CBs	20.075	157.703	82.693	68.697	48.665	∑CBs	20.84	86.15	58.70	61.06	19.66

Table 22 continued**:** PCB concentrations (ng g^{-1} wm) in eggs of various wild bird species

Species	Min	Max	Mean	Median	SD
Insectivores/Omnivores: *Cattle Egret (n = 6)*					
%Lipid	5.81	8.59	6.89	6.52	1.01
CB28	0.02	0.15	0.05	0.03	0.05
CB31	0.03	0.04	0.02	0.02	0.01
CB47	0.03	0.12	0.07	0.05	0.04
CB52	0.07	0.11	0.08	0.08	0.01
CB56	ND	ND	0.01	0.01	0.00
CB66	0.02	0.21	0.10	0.08	0.08
CB74	0.02	0.21	0.08	0.04	0.09
CB99	0.02	0.36	0.13	0.05	0.15
CB101	0.02	0.04	0.03	0.03	0.01
CB105	0.01	0.34	0.12	0.04	0.15
CB110	ND	0.10	0.04	0.02	0.03
CB114	ND	0.02	0.01	0.00	0.01
CB118	0.07	1.66	0.55	0.22	0.65
CB123	ND	0.05	0.02	0.01	0.02
CB128	ND	0.41	0.10	0.01	0.16
CB137	ND	0.05	0.02	0.01	0.02
CB138	0.09	5.72	1.48	0.30	2.25
CB141	ND	0.04	0.01	0.01	0.01
CB149	ND	0.35	0.07	0.01	0.14
CB151	ND	0.13	0.03	0.01	0.05
CB153	0.34	12.87	3.70	1.14	5.02
CB156	0.02	1.09	0.31	0.08	0.43
CB157	0.01	0.18	0.06	0.02	0.07
CB167	0.03	0.55	0.17	0.07	0.21
CB170	0.04	3.18	0.88	0.23	1.26
CB180	0.21	9.01	2.77	0.83	3.65
CB183	0.02	1.23	0.36	0.08	0.50
CB187	0.04	2.43	0.77	0.13	1.08
CB189	0.01	0.21	0.06	0.02	0.08
CB194	0.07	3.33	1.10	0.31	1.43
CB196	0.05	5.73	1.54	0.21	2.35
CB199	ND	0.09	0.03	0.01	0.03
CB206	0.07	4.88	1.63	0.31	2.23
CB209	ND	0.46	0.20	0.09	0.17
∑CBs	1.388	55.351	16.508	4.298	22.347
Insectivores/Omnivores: *Crowned Lapwing (n = 6)*					
%Lipid	8.71	15.51	11.48	11.12	2.21
CB28	0.07	1.46	0.35	0.11	0.55
CB31	ND	ND	0.01	0.01	0.00
CB47	0.08	0.82	0.25	0.12	0.29
CB52	0.12	0.50	0.19	0.13	0.15
CB56	ND	ND	0.01	0.01	0.00
CB66	0.10	2.05	1.02	0.80	0.81
CB74	ND	1.15	0.30	0.13	0.43
CB99	0.15	2.09	0.91	0.87	0.69
CB101	0.13	1.70	0.49	0.23	0.60
CB105	0.12	2.08	0.63	0.42	0.73
CB110	ND	3.27	0.76	0.24	1.25
CB114	0.02	0.17	0.07	0.06	0.05
CB118	1.88	6.57	2.85	2.03	1.84
CB123	ND	0.20	0.08	0.06	0.07
CB128	0.10	1.27	0.42	0.26	0.44
CB137	0.04	0.57	0.22	0.17	0.20
CB138	1.02	14.88	5.27	2.94	5.35
CB141	0.02	0.31	0.08	0.04	0.11
CB149	0.26	3.12	1.18	0.44	1.28
CB151	ND	0.44	0.13	0.04	0.17
CB153	1.91	24.15	9.17	5.26	8.96
CB156	0.15	2.15	0.69	0.39	0.75
CB157	0.05	0.67	0.21	0.16	0.23
CB167	0.14	1.33	0.49	0.37	0.43
CB170	0.38	4.90	1.72	0.98	1.70
CB180	1.08	14.58	4.88	2.95	5.05
CB183	0.30	3.40	1.17	0.72	1.17
CB187	0.75	10.53	4.30	3.09	3.81
CB189	0.03	0.38	0.12	0.07	0.13
CB194	0.47	3.84	2.19	2.28	1.28
CB196	0.19	6.28	2.21	1.71	2.24
CB199	0.02	0.29	0.11	0.09	0.09
CB206	0.14	9.02	3.09	2.31	3.26
CB209	ND	1.66	0.62	0.56	0.56
∑CBs	10.09	125.86	46.12	31.09	43.01

Species	Min	Max	Mean	Median	SD
Insectivores/Omnivores: *Red-knobbed Coot (n = 6)*					
%Lipid	12.43	14.11	13.28	13.26	0.54
CB28	0.41	3.95	1.42	0.99	1.38
CB31	0.05	0.82	0.35	0.26	0.32
CB47	0.10	1.02	0.38	0.27	0.36
CB52	0.08	0.65	0.32	0.29	0.24
CB56	ND	ND	0.01	0.01	0.00
CB66	0.29	2.56	1.05	0.84	0.90
CB74	0.22	1.62	0.65	0.51	0.54
CB99	0.13	0.73	0.42	0.41	0.30
CB101	0.06	0.45	0.27	0.29	0.17
CB105	0.21	0.81	0.52	0.51	0.29
CB110	0.05	0.40	0.18	0.16	0.14
CB114	ND	0.09	0.04	0.04	0.04
CB118	0.53	2.07	1.30	1.32	0.74
CB123	0.03	0.12	0.07	0.07	0.04
CB128	0.06	0.34	0.18	0.16	0.13
CB137	0.02	0.07	0.04	0.04	0.03
CB138	0.55	3.29	1.90	1.81	1.22
CB141	ND	0.07	0.03	0.03	0.02
CB149	0.06	0.52	0.28	0.24	0.20
CB151	0.04	0.28	0.14	0.10	0.11
CB153	0.49	3.21	1.63	1.27	1.17
CB156	0.10	0.39	0.24	0.23	0.11
CB157	0.03	0.09	0.06	0.06	0.03
CB167	0.08	0.30	0.16	0.15	0.08
CB170	0.11	0.52	0.35	0.37	0.17
CB180	0.27	1.19	0.79	0.82	0.36
CB183	0.07	0.31	0.21	0.22	0.09
CB187	0.15	0.96	0.53	0.52	0.29
CB189	0.01	0.03	0.02	0.02	0.01
CB194	0.22	0.71	0.42	0.40	0.16
CB196	0.10	0.52	0.23	0.18	0.15
CB199	0.01	0.03	0.02	0.02	0.01
CB206	0.07	0.57	0.20	0.14	0.19
CB209	ND	ND	0.09	0.09	0.00
∑CBs	4.94	26.92	14.42	13.30	8.79
Granivores: *Cape Sparrow (n = 9: 3 pools of 3 eggs)*					
%Lipid	3.85	7.14	5.84	6.54	1.75
CB28	ND	0.07	0.03	0.03	0.03
CB31	ND	0.04	0.03	0.04	0.02
CB47	0.08	0.12	0.09	0.08	0.02
CB52	0.09	0.15	0.12	0.11	0.03
CB56	ND	ND	0.01	0.01	0.00
CB66	0.04	0.11	0.07	0.06	0.04
CB74	0.05	0.12	0.08	0.07	0.03
CB99	0.06	0.40	0.18	0.08	0.19
CB101	0.08	0.52	0.24	0.11	0.25
CB105	ND	0.12	0.11	0.05	0.12
CB110	0.03	0.25	0.07	0.06	0.05
CB114	ND	ND	0.00	0.00	0.00
CB118	0.29	1.86	1.25	1.61	0.84
CB123	ND	0.06	0.02	0.01	0.03
CB128	0.03	0.42	0.17	0.05	0.22
CB137	0.02	0.09	0.05	0.04	0.04
CB138	0.26	4.85	1.90	0.57	2.56
CB141	0.01	0.12	0.05	0.02	0.06
CB149	ND	0.47	0.19	0.10	0.24
CB151	ND	0.08	0.04	0.01	0.04
CB153	1.27	11.69	6.96	7.93	5.28
CB156	0.04	0.63	0.25	0.10	0.33
CB157	0.01	0.11	0.05	0.02	0.06
CB167	0.05	0.39	0.18	0.09	0.19
CB170	0.13	1.33	0.60	0.33	0.64
CB180	0.33	2.32	1.14	0.76	1.05
CB183	0.08	0.64	0.29	0.17	0.30
CB187	0.14	1.28	0.59	0.36	0.60
CB189	0.00	0.07	0.03	0.02	0.03
CB194	0.16	0.60	0.40	0.42	0.22
CB196	0.08	0.30	0.20	0.22	0.11
CB199	0.03	0.04	0.04	0.04	0.01
CB206	0.09	0.29	0.19	0.19	0.10
CB209	ND	0.52	0.23	0.09	0.25
∑CBs	5.78	25.29	15.76	16.19	9.76

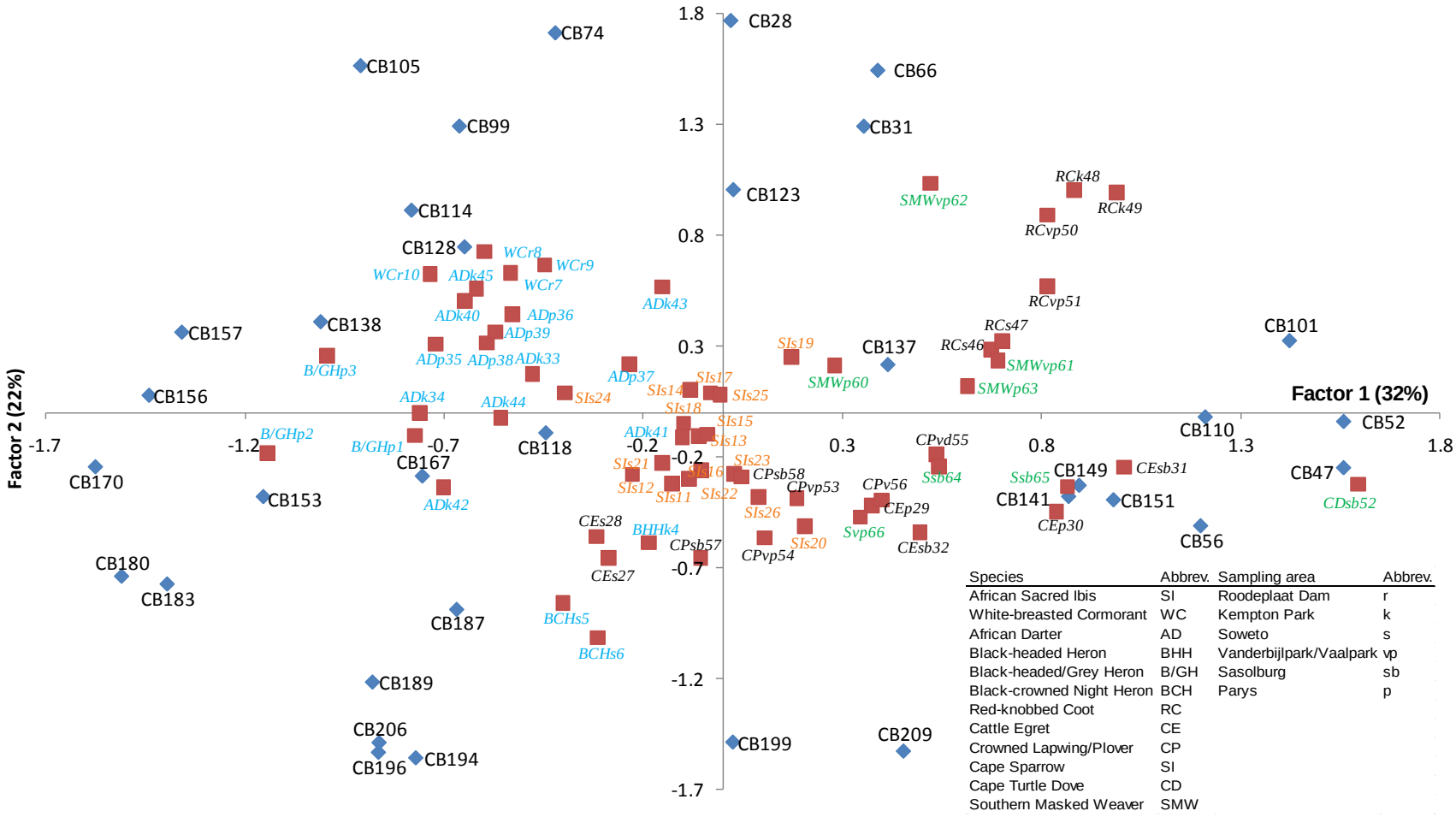
Table 22 continued**:** PCB concentrations (ng g^{-1} wm) in eggs of various wild bird species

Species	Min	Max	Mean	Median	SD	Species	
Granivores: *Southern Masked Weaver (n = 8: 4 pools of 2 eggs)*						Granivores: *Cape Turtle Dove (n = 3: 1 pool of 3 eggs)*	
%Lipid	2.97	6.06	4.46	4.40	1.29	%Lipid	8.52
CB28	0.09	4.75	1.30	0.19	2.30	CB28	0.01
CB31	0.03	0.38	0.13	0.05	0.17	CB31	0.01
CB47	0.04	2.76	0.77	0.15	1.32	CB47	0.17
CB52	0.12	0.31	0.18	0.15	0.09	CB52	0.04
CB56	ND	0.00	0.01	0.01	0.00	CB56	0.01
CB66	0.24	6.52	1.90	0.41	3.09	CB66	0.01
CB74	0.31	7.06	2.06	0.45	3.33	CB74	0.01
CB99	0.30	4.63	1.50	0.53	2.10	CB99	0.01
CB101	0.22	2.95	1.02	0.45	1.31	CB101	0.02
CB105	0.17	5.83	1.70	0.39	2.76	CB105	0.00
CB110	ND	0.88	0.30	0.14	0.40	CB110	0.02
CB114	ND	0.45	0.12	0.00	0.22	CB114	0.00
CB118	0.37	1.30	0.58	0.50	0.55	CB118	0.07
CB123	ND	0.49	0.15	0.04	0.23	CB123	0.01
CB128	0.10	1.89	0.59	0.20	0.87	CB128	0.00
CB137	0.04	0.65	0.20	0.07	0.30	CB137	0.01
CB138	0.70	2.86	1.43	1.08	0.97	CB138	0.06
CB141	0.02	0.33	0.12	0.06	0.15	CB141	0.01
CB149	0.07	1.18	0.45	0.27	0.51	CB149	0.01
CB151	ND	0.02	0.02	0.02	0.01	CB151	0.01
CB153	0.94	13.04	5.42	3.85	5.47	CB153	0.12
CB156	0.12	3.05	0.97	0.35	1.40	CB156	0.00
CB157	0.04	0.84	0.26	0.07	0.39	CB157	0.00
CB167	0.11	1.03	0.41	0.26	0.43	CB167	0.02
CB170	0.17	2.66	1.05	0.68	1.13	CB170	0.01
CB180	0.40	4.79	2.02	1.44	1.98	CB180	0.06
CB183	0.10	0.89	0.43	0.36	0.36	CB183	0.01
CB187	0.19	1.49	0.80	0.75	0.61	CB187	0.02
CB189	0.01	0.18	0.07	0.04	0.08	CB189	0.00
CB194	0.26	1.99	0.90	0.67	0.79	CB194	0.04
CB196	0.19	0.81	0.43	0.36	0.28	CB196	0.02
CB199	ND	0.06	0.03	0.03	0.02	CB199	0.01
CB206	0.18	1.09	0.46	0.29	0.42	CB206	0.02
CB209	ND	0.16	0.11	0.09	0.04	CB209	0.09
∑CBs	5.97	74.30	27.75	15.36	31.83	∑CBs	0.92

ND: not detected

4.3.1. Tri- to deca-CB in wild bird eggs

For further data analysis PCBs were divided into eight groups according to the number of chlorine atoms: tri-CB (CB-28, -31), tetra-CB (CB-47, -52, -56, -66, -74), penta-CB (CB-99, -101, -105, -110, -114, -118, -123), hexa-CB (CB-128, -137, -138, -141, -149, -151, -153, -156, -157, -167), hepta-CB (CB-170, -180, -183, -187, -189), octa-CB (CB-194, -196, -199), nona-CB (CB-206), and deca-CB (CB-209), as well as five groups according to metabolic potential and three groups according to the main MFO induction as described in section 2.2.7 (Table 11). One-way ANOVA results (log-transformed data) indicated significant differences between species analysed ($p = 0.00$). Hexa-CB was the predominant class of PCBs in all species contributing between 28 - 71% to ΣPCBs measured followed by hepta-CB in Cattle Egrets, Crowned Lapwing, African Darter, White-breasted Cormorant, Heron *spp*., and the African Sacred Ibis contributing between 20 - 28% to ΣPCBs (Table 23). For the Red-knobbed Coot and Cape Sparrow, the second most abundant group was penta-CB, and for the Cape Turtle Dove tetra-CB. Tri-CB and deca-CB were the groups with the lowest contribution (0.4 – 3%), with the exception of the Cape Turtle Dove where deca-CB contributed 10% to ΣPCBs (Table 23).



Species	Abbrev.	Sampling area	Abbrev.
African Sacred Ibis	SI	Roodeplaat Dam	r
White-breasted Cormorant	WC	Kempton Park	k
African Darter	AD	Soweto	s
Black-headed Heron	BHH	Vanderbijlpark/Vaalpark	vp
Black-headed/Grey Heron	B/GH	Sasolburg	sb
Black-crowned Night Heron	BCH	Parys	p
Red-knobbed Coot	RC		
Cattle Egret	CE		
Crowned Lapwing/Plover	CP		
Cape Sparrow	SI		
Cape Turtle Dove	CD		
Southern Masked Weaver	SMW		

Figure 35: Biplot of the principle component analysis for PCBs for all wild bird species. The African Sacred Ibis, the only scavenging species, is indicated in orange, piscivores in blue, insectivores/omnivores in black and granivores in green. Data labels for individual bird eggs used as listed in Tables 14-18

Table 23: Percentage contribution of tri- to deca-CB to ΣPCB measured in wild bird eggs from South Africa

Species PCB group	Cattle Egret	Crowned Lapwing	Red-knobbed Coot	African Darter	White-breasted Cormorant	Heron *spp.*	Cape Turtle Dove	Cape Sparrow	Southern Masked Weaver	African Sacred Ibis
Tri-CB	1	0.5	10	1	3	1	1	0.5	2	1
Tetra-CB	5	4	14	3	7	1	26	2	7	3
Penta-CB	8	13	22	14	15	7	15	12	16	9
Hexa-CB	36	33	33	47	47	35	28	71	45	35
Hepta-CB	29	26	15	26	22	20	10	9	21	32
Octa-CB	11	14	5	6	4	18	8	4	7	12
Nona-CB	7	8	1	3	2	16	3	1	2	7
Deca-CB	2	2	1	0.2	0.2	2	10	0.5	1	1

There was also a statistically significant difference between the various feeding guilds when looking at tri- to deca-PCB (one-way ANOVA, log transformed data, $p = 0.00$). Piscivores had significantly higher levels of tri- to deca-CB ($p < 0.05$). The level of tri-CB was significantly higher in scavengers compared to granivores, and for penta-CB compared to insectivores/omnivores ($p < 0.05$). Hexa-CB and hepta-CB differed significantly between all guilds ($p < 0.05$), with the exception of granivores and insectivores/omnivores ($p = 0.99$ and $p = 0.53$, respectively) (Figure 36). Scavengers also had statistically significantly higher concentrations for octa-, nona- and deca-CB when compared to insectivores/omnivores and granivores ($p < 0.05$) (Figure 36).

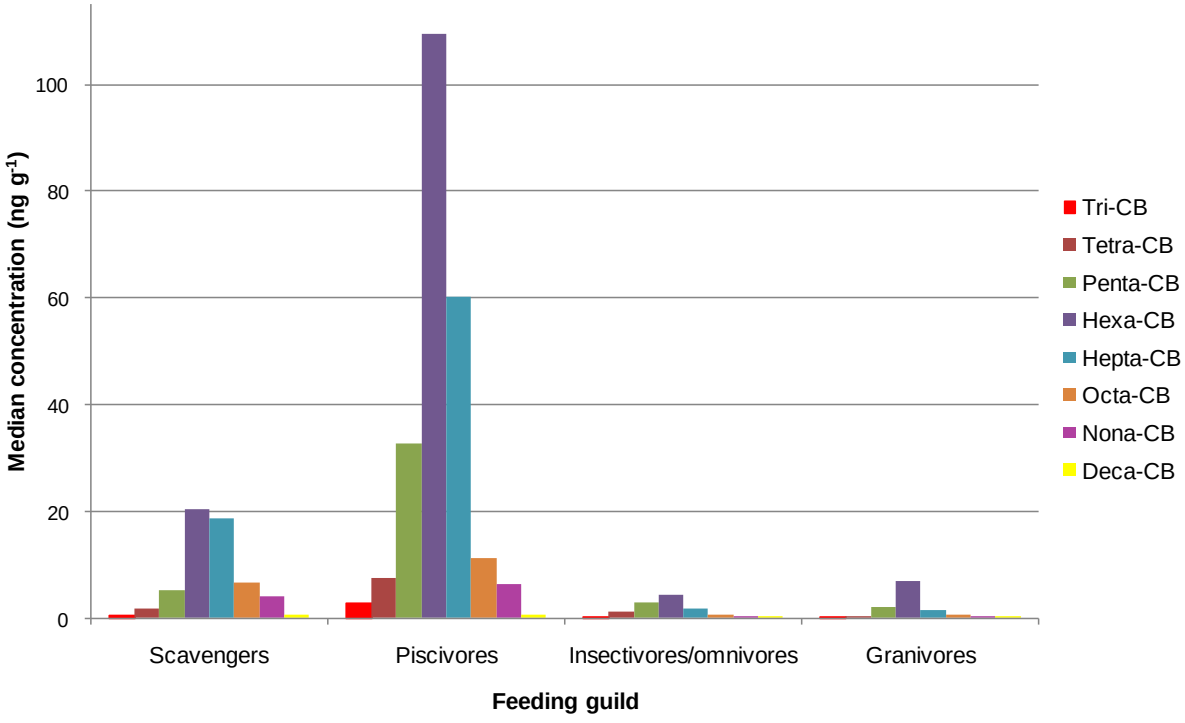


Figure 36: The concentration of tri- to deca-CB between the various feeding guilds

PCB groups also differed statistically significantly between the different feeding habitat preferences (one-way ANOVA, log transformed data, $p < 0.0001$). Tri- and tetra-CB differed significantly between all feeding habitats ($p < 0.05$). For penta-, hexa-, hepta-, octa-, nona- and deca-CB, terrestrial species eggs had significantly lower concentrations than eggs from species utilising aquatic and combined habitats (Figure 37).

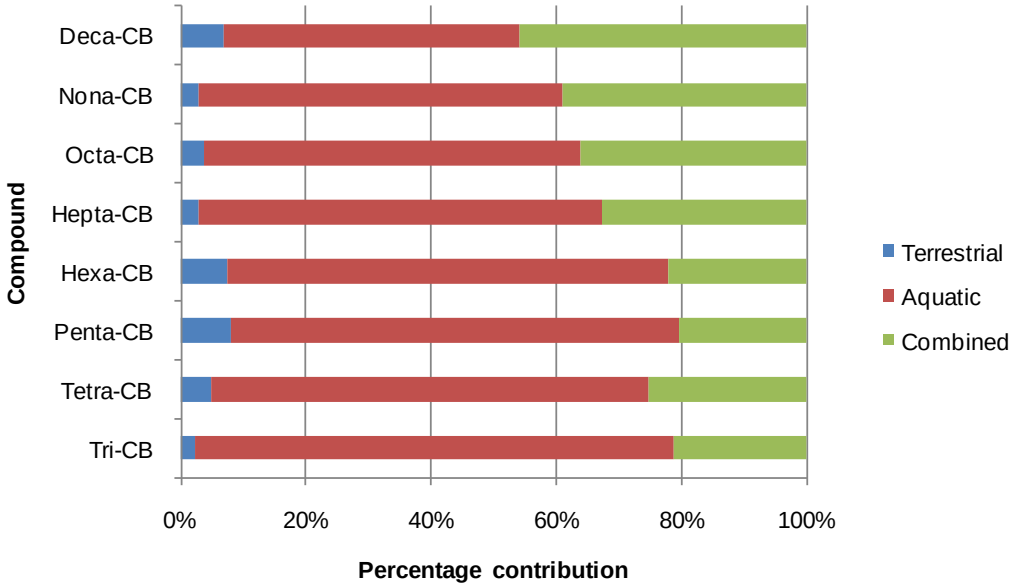


Figure 37: Contribution of tri- to deca-CB to the ΣPCB in terrestrial, aquatic, and combined habitats

4.3.2. Metabolic and MFO-induction PCB groups

Metabolic groups as well as PB-type and mixed type inducers and DL-PCBs differed significantly between species, guild and feeding habitat types (one-way ANOVA log transformed data, $p < 0.05$). Group I PCBs differed significantly ($p < 0.05$) between the African Darter and African Sacred Ibis, Cattle Egret, Red-knobbed Coot, Crowned Plover, Cape Sparrow, Southern Masked Weaver, and Heron *spp*. as well as between the White-breasted Cormorant and the African Sacred Ibis, Cattle Egret and Red-knobbed Coot (Figure 38). Group II PCBs differed significantly ($p < 0.05$) between the African Darter and White-breasted Cormorant and all other species, although these two species did not differ significantly from each other (Figure 38). For Group III PCBs, the White-breasted Cormorant differed significantly ($p < 0.05$) from all species with the exception of the African Darter, and the African Darter differed from all remaining species with the exception of the Cape Turtle Dove (Figure 38). Groups IV and V show no statistically significant difference between the various species.

The African Darter and the White-breasted Cormorant had significantly higher levels of DL-PCBs (mono-ortho) and mixed type inducer compared to all other species ($p < 0.05$). However, for mixed type inducers the African Darters, White-breasted Cormorant, and Cape Turtle Dove did not differ significantly from each other ($p > 0.05$) (Figure 39). For PB-type inducers the African Darter had significantly higher levels than the African Sacred Ibis, Cattle Egret, Crowned Lapwing, Red-knobbed Coot, Southern Masked Weaver and Heron *spp*. Additionally, the White-breasted Cormorant had significantly higher levels of PB-type inducers compared to the African Sacred Ibis, Cattle Egret Crowned Lapwing, Red-knobbed Coot and Heron *spp* ($p < 0.05$) (Figure 39).

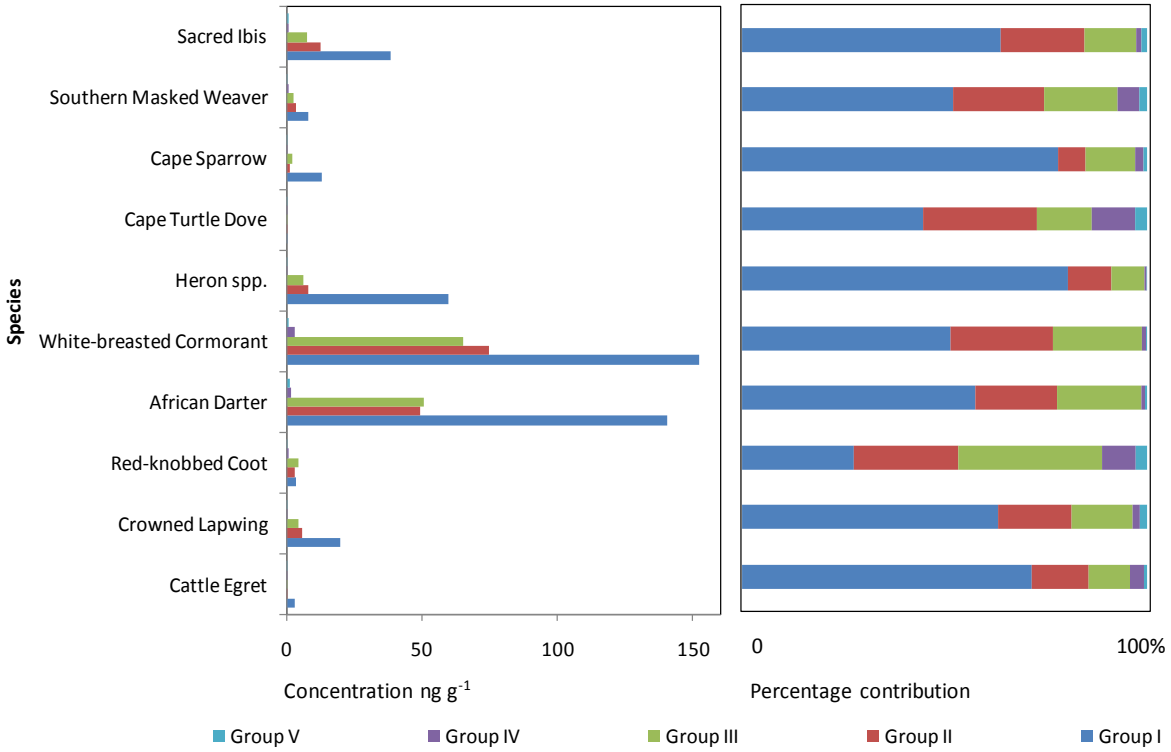


Figure 38: The concentration and percentage contribution of PCB metabolic groups in various species of wild bird

The one-way ANOVA results indicated that the piscivore guild and consequently birds that fed in the aquatic habitat, had significantly higher levels of metabolic groups I, II and III as well as DL-PCBs, PB-type and mixed inducers ($p < 0.05$). None of the other eggs grouped in guilds or feeding habitats differed statistically significantly from one another and group VI and V did not differ statistically significantly between the various guilds or feeding habitats ($p > 0.05$).

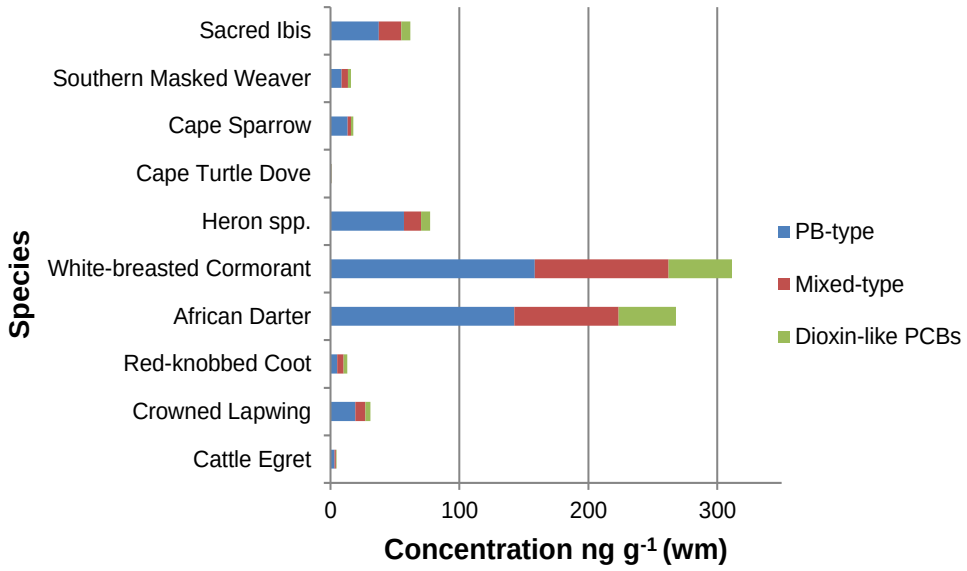


Figure 39: Levels of PCB groups, based on enzyme induction, in wild bird species from South Africa

4.3.3 PCBs and between PCBs and eggshell thickness

To investigate the relationship between individual PCBs, a product-moment correlation on log-transformed data was used (Figure 40). While PCB congeners were strongly correlated with one another, CB-47 showed the lowest frequency of correlation to other congeners (40%), and CB-138 and -114 showed the highest frequency of correlation being statistically significantly correlated to 94% of the PCB congeners while CB-74, -128, -180, -170, -105, -123, -156, -157 was correlated to > 90% of the PCB congeners (Figure 40).

To assess the possible effect of PCBs on eggshell thinning a Spearman rank order correlation was performed. The correlations were confined to the metabolic groupings, DL-PCB and the MFO-induction PCB groups. Due to smaller sample size, only non-parametric correlation analysis was performed. Only two species showed a statistically significant correlation ($p < 0.05$) between the various PCB groups and the eggshell thinning, the Crowned Lapwing and the combined granivore species. The Crowned Lapwing showed a positive correlation with all groups analysed except group IV, with r varying between 0.94 - 0.88. However, the granivores showed a negative correlation with group IV PCBs (r = -0.7).

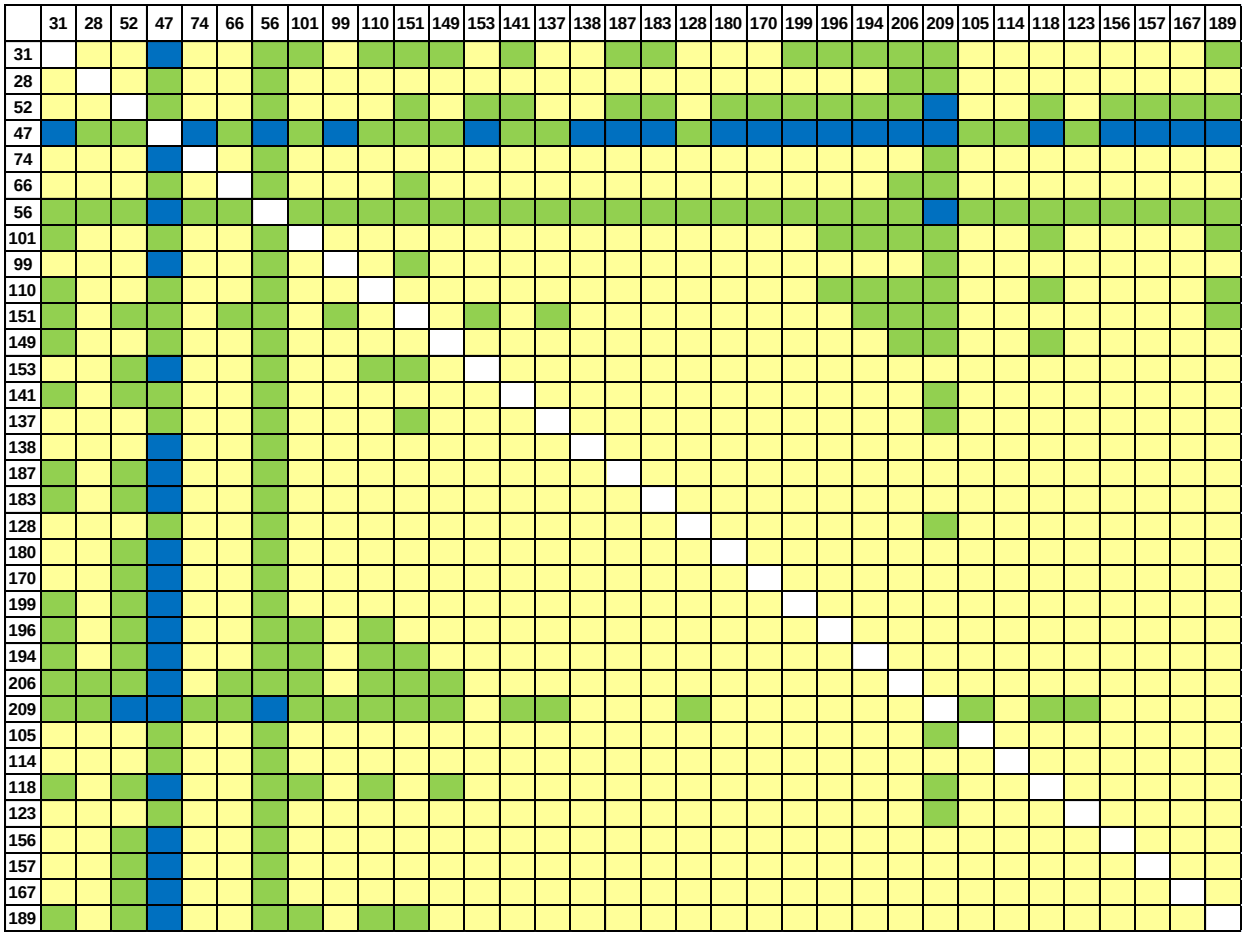


Figure 40: Correlation matrix for individual PCB congeners, blue indicates no significant correlation ($p > 0.05$), green a significant correlation ($p < 0.05$) with $r < 0.6$, and yellow a statistically significant correlation ($p < 0.05$) with $r > 0.6$

4.4 ORGANOHALOGEN COMPOUNDS IN WILD BIRD EGGS FROM HIGHLY INDUSTRIALISED AREAS OF CENTRAL SOUTH AFRICA

An individual is exposed to a mixture of POPs, including BFRs, OCPs and PCBs. Due to differences in chemical characteristics, the differences in concentration and distribution of halogenated compounds can be used to derive information on the exposure of species, guilds and habitats to these compounds. The differences in distribution and occurrence of organohalogens were investigated using PCA and one-way ANOVA's (log transformed data).

4.4.1. The occurrence of organohalogen compounds in the different bird species studied

The concentration of ΣOCPs was higher than ΣBFRs and ΣPCBs in all species, with the exception of the African Sacred Ibis and granivores (Figure 41). The levels of all three pollutant groups were similar in the African Sacred Ibis, with ΣPCBs having the highest concentration. However, in granivores ΣBFRs had the highest concentration (Figure 41).

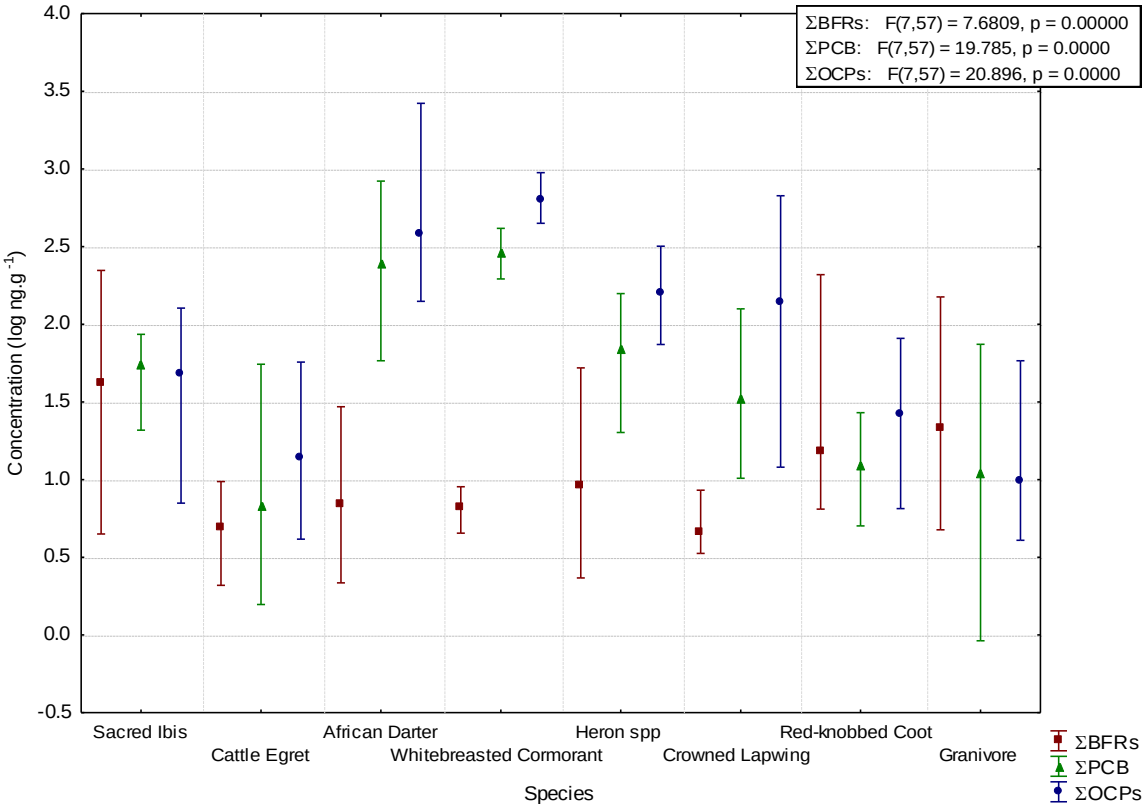


Figure 41: Mean concentration of organohalogenated compounds in various species collected. Whiskers show minimum and maximum values. The one-way ANOVA f and p values for log-transformed data are displayed in the inset. All granivores were combined due to small sample sizes of individual species

One-way ANOVA results indicated ΣBFRs levels in the African Sacred Ibis differed statistically significantly from the Cattle Egret, African Darter, White-breasted Cormorant, Crowned Lapwing and Heron *spp.* ($p < 0.05$), while granivores differed statistically significantly from the Crowned Lapwing. In the case of ΣPCBs, there was a large variation between species. Species that did not differ statistically significantly from each other ($p > 0.05$) were:

- the African Sacred Ibis, Crowned Lapwing and Heron *spp.*;
- the Cattle Egret, Red-knobbed Coot and granivores; African Darter, White-Breasted Cormorant and heron *spp.*;
- Crowned Lapwing, Red-knobbed Coot, Granivore and Heron *spp.*;
- Red-knobbed Coot and Heron *spp*.

When looking at the cluster arrangement of the groups within the various species, all species showed the same cluster arrangement with the exception of the Cape Sparrow (Figure 42). For the Cape Sparrow the two main clusters consisted of ΣOCPs against a combined cluster of ΣPCBs and ΣBFRs, whereas for all other species two main clusters consisted of ΣBFRs against a combined cluster of ΣPCBs and ΣOCPs

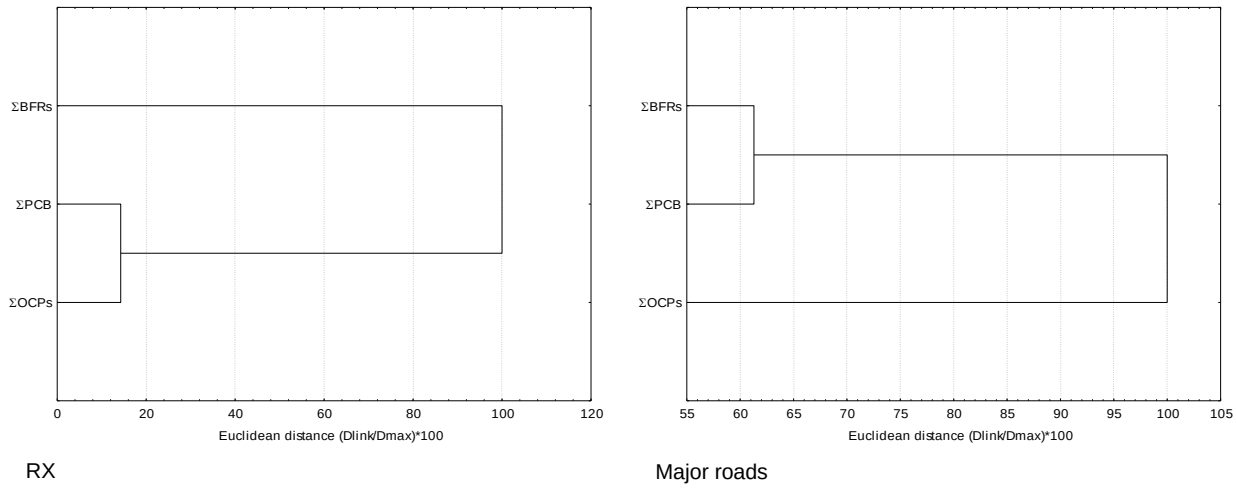
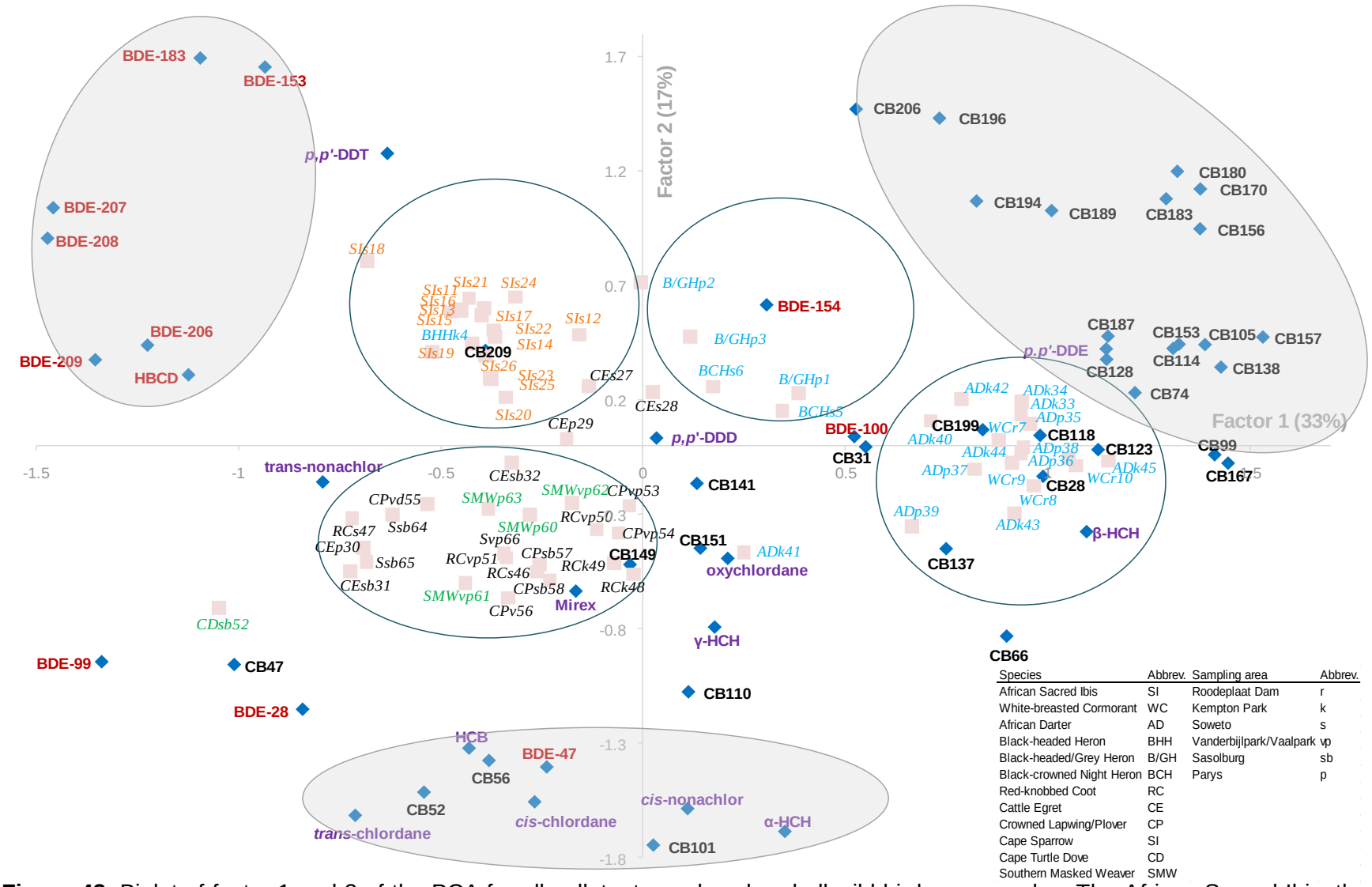


Figure 42: Cluster analysis of species and the main halogenated pollutant groups analysed

The distribution of the various pollutants between bird species was additionally investigated through PCA analysis of transformed data. For this PCA analysis, all individual samples and all pollutants analysed were included (Figure 43 and 44). Only the first three factors will be discussed. All factor scores and loadings are given in the supplementary data (Table S10 and S11).



Species	Abbrev.	Sampling area	Abbrev.
African Sacred Ibis	SI	Roodeplaat Dam	r
White-breasted Cormorant	WC	Kempton Park	k
African Darter	AD	Soweto	s
Black-headed Heron	BHH	Vanderbijlpark/Vaalpark	vp
Black-headed/Grey Heron	B/GH	Sasolburg	sb
Black-crowned Night Heron	BCH	Parys	p
Red-knobbed Coot	RC		
Cattle Egret	CE		
Crowned Lapwing/Plover	CP		
Cape Sparrow	SI		
Cape Turtle Dove	CD		
Southern Masked Weaver	SMW		

Figure 43: Biplot of factor 1 and 2 of the PCA for all pollutants analysed and all wild bird egg samples. The African Sacred Ibis, the only scavenging species, is indicated in orange, piscivores in blue, insectivores/omnivores in black and granivores in green. Data labels for individual bird eggs used as listed in Tables 14-18. Pollutants are indicated as follows: OCPs in purple, PCBs in black, and BDEs in red

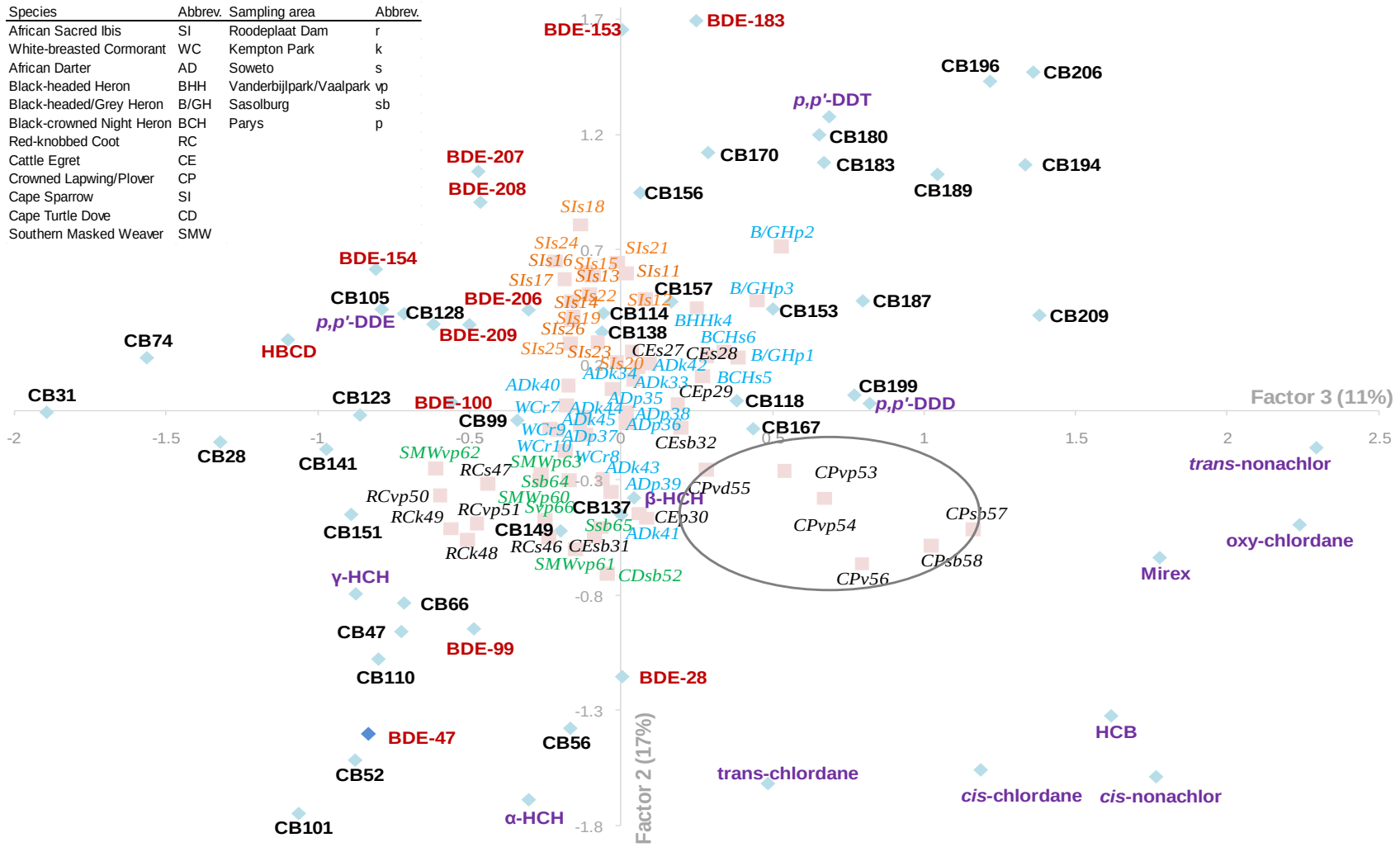


Figure 44: Biplot of factor 2 and factor 3 for the PCA of all pollutants analysed and all wild bird egg samples. The African Sacred Ibis, the only scavenging species, is indicated in orange, piscivores in blue, insectivores/omnivores in black and granivores in green. Data labels for individual bird eggs used as listed in Tables 14-18. Pollutants are indicated as follows: OCPs in purple, PCBs in black, and BDEs in red

Factor 1 explained 34% of the variance in the dataset, followed by factor 2 with 17% and factor 3 with 11%. Factor 1 was a contrast between BDEs, with negative scores, and PCBs, with positive scores (Figure 43). Factor 2 was a contrast between a mixture of OCPs, PCBs and BDE-47 with negative loadings and a mixture of PCBs, BDEs and *p,p'*-DDT with positive scores. The OCPs and PCBs responsible for the contrast in factor 2 with negative scores consisted of chlordanes, α-HCH and HCB as well as the lower chlorinated/brominated PCBs and BFRs. The positive scores were a mixture of the higher chlorinated/brominated PCBs and BFRs, as well as the OCP *p,p'*-DDT (Figures 43 and 44). Factor 3 was a contrast between PCBs and HBCD with negative scores and OCPs, and higher chlorinated PCBs with positive scores.

The granivores, insectivores and omnivores with negative loadings and the African Darter and White-breasted Cormorant with positive loadings were distributed along factor 1. Granivores, insectivores and omnivores with negative loadings and the African Sacred Ibis and Heron *spp*. were distributed along factor 2 (Figures 43 and 44). The Southern Masked Weaver and the Red Knobbed Coot with negative loadings and the Crowned Lapwing with positive loadings were distributed along factor 3 (Figure 44). No distinct separation of sampling sites could be seen in any of the biplots.

4.4.2. The occurrence of organohalogen compounds in the different feeding guilds

The pattern of the various organohalogen compounds differed between the different feeding guilds. The patterns in the feeding guilds were as follows: for scavenger PCBs>OCPs>BFRs, for piscivores OCPs>PCBs>BFRs, for insectivores/omnivores OCPs>PCBs>BFRs, and for granivores BFRs>PCBs>OCPs (Figure 45). These patterns were further investigated using a one-way ANOVA and post hoc Tukey test with log transformed data. There was a statistically significant difference in the concentration of the various pollutant classes between the feeding guilds ($p = 0.00$) (Figure 45). For ΣBFRs, scavengers and granivores had significantly higher levels ($p < 0.05$) compared to all other guilds except each another ($p = 0.36$). In the case of ΣPCBs, scavengers and piscivores had significantly higher concentration compared to all other species, with piscivore having statistically significantly higher levels than scavengers ($p < 0.05$). PCB concentrations in insectivores/omnivores and granivores did not differ statistically significantly from one another ($p = 0.92$). For ΣOCPs, all guilds differed statistically significantly from each other ($p < 0.05$) except for scavengers and insectivores/omnivores ($p = 0.9$).

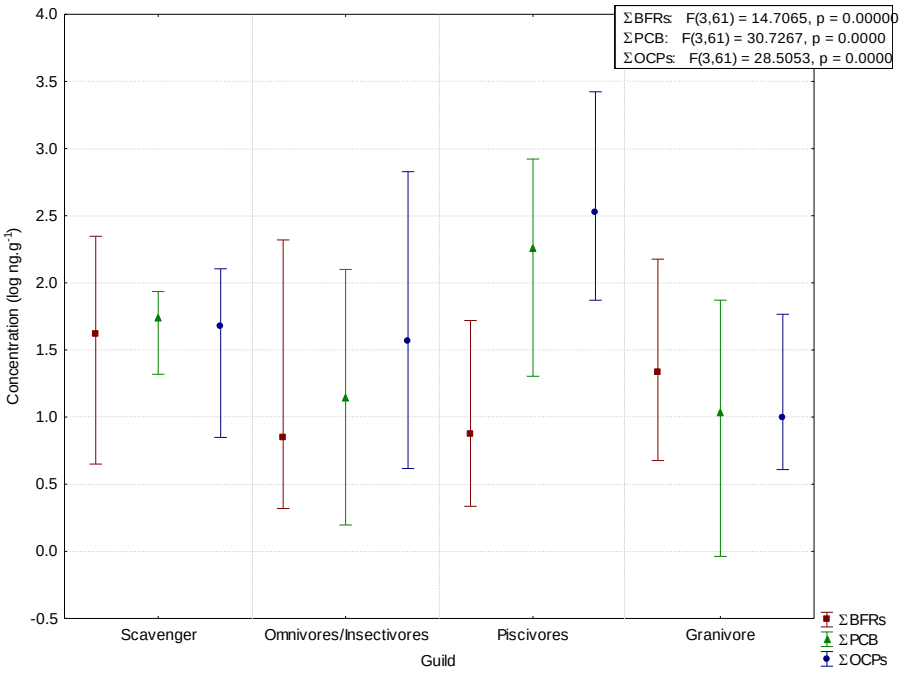


Figure 45: Concentration of organohalogens in the various feeding guilds. Whiskers show minimum and maximum values

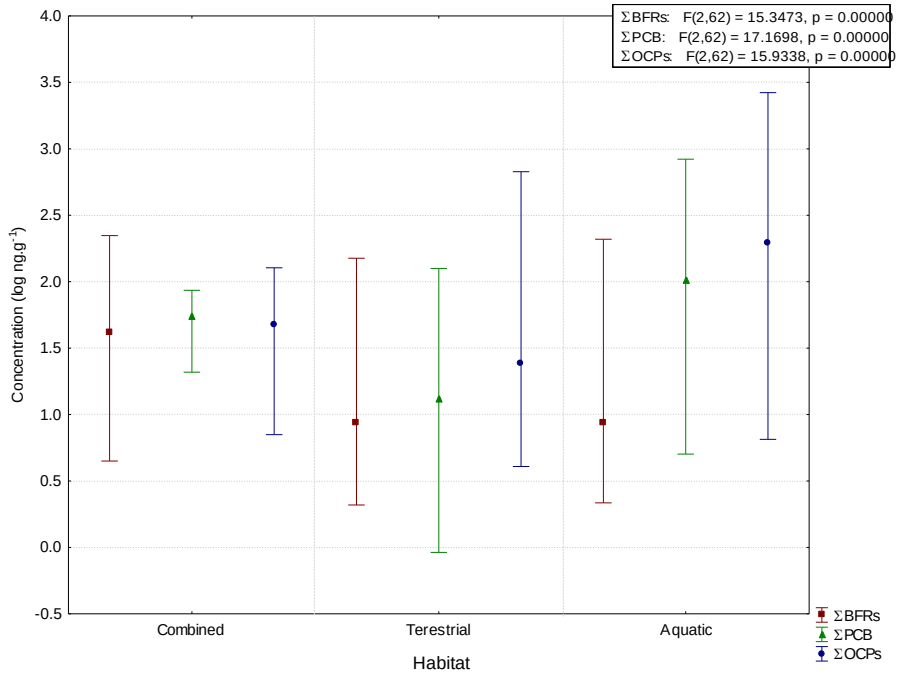


Figure 46: Concentration of organohalogens according to feeding habitat preference. Whiskers show minimum and maximum values

4.4.3. The occurrence of organohalogen compounds in the different feeding habitats

The pattern of the various organohalogen compounds differed between the different feeding habitats as with the feeding guilds. For scavengers, as the only combined habitat feeders, the pattern was as stated in 4.4.2: PCBs>OCPs>BFRs. For terrestrial birds and aquatic birds the pattern was OCPs>PCBs>BFRs (Figure 46).

There were significant differences of organohalogen compounds between the various feeding habitats (one-way ANOVA on log-transformed data, $p < 0.0001$). For ΣBFRs the combined feeding habitat birds (African Sacred Ibis) had significantly higher levels ($p < 0.05$). In the case of ΣPCBs, the terrestrial birds had significantly lower concentrations ($p < 0.05$) and for ΣOCPs, the aquatic birds had significantly higher concentration compared to all other habitat usages ($p < 0.05$).

4.4.4. Ratios between organohalogen compound groups

Ratios of the various compounds can elucidate information pertaining to differences in environmental input and sources (Hughes *et al*., 1998; Pulkrabová *et al*., 2007; Bouwman *et al*., 2008). The ratio analysis of DDE/ΣPCB is used to distinguish the predominant source for an area, either agricultural or industrial. DDE is used instead of DDT, since DDT is degraded to DDE in the environment. To distinguish between recent and historic environmental, the ΣPBDE/ΣPCB ratio is used. Ratios are summarised in Table 24 and the sample specific values are included in the supplementary data (Table S12).

The ratio of DDE/ΣPCB ranged between 0.03 - 11 (Table S12). The highest median ratios were found in Kempton Park for the Heron *spp*., with the lowest ratios in Sasolburg and Vanderbijlpark in the Crowned Lapwing (Table 24). The ratio of ΣPBDE/ΣPCB ranged between 0.01 - 27 (Table S12). High median ratios were found in the Red-Knobbed Coot and the granivores (14 and 12, respectively). All other median ratio values were between 0.02 and 3. The areas with the highest ratios were Soweto and Vanderbijlpark (Table 24).

The differences between species, guilds, feeding habitat types, and sampling areas were investigated using a one-way ANOVA and post hoc Tukey test. The ratio of DDE/ΣPCB differed statistically significantly between species ($p = 0.04$), guilds ($p = 0.03$), and feeding habitat types ($p = 0.00$). The difference between the guilds was mainly due to differences between piscivores and scavengers ($p = 0.03$). Aquatic birds had higher ratios than both the combined ($p = 0.009$) and terrestrial birds ($p = 0.02$). The sampling areas differed statistically

significantly from one another (p = 0.00) where the ratio was statistically significantly higher in Kempton Park compared to Soweto (p = 0.004) and Vanderbijlpark (p = 0.02).

The ΣPBDE/ΣPCB ratio did not differ statistically significantly between species, sampling area, or feeding habitat (p > 0.05). However, the ratio did differ between the various guilds (p = 0.03), where the granivores were statistically significantly higher than the piscivores (p = 0.02).

Table 24: Ratios of DDE/ΣPCB and ΣPBDE/ΣPCB in different bird species according to habitat and sampling area

Species	Guild	Habitat	Sampling area	Ratio DDE/ΣPCB	Ratio ΣPBDE/ ΣPCB
African Sacred Ibis	**Scavenger**	**Combined**	**Soweto**	**0.62**	**0.70**
Cattle Egret	Omnivores/Insectivores	Terrestrial	Soweto	0.96	0.13
Cattle Egret	Omnivores/Insectivores	Terrestrial	Parys	3.04	2.78
Cattle Egret	Omnivores/Insectivores	Terrestrial	Sasolburg	1.86	3.25
Cattle Egret	**Omnivores/Insectivores**	**Terrestrial**		**1.21**	**0.46**
African Darter	Piscivores	Aquatic	Kempton Park	1.43	0.02
African Darter	Piscivores	Aquatic	Parys	1.01	0.02
African Darter	**Piscivores**	**Aquatic**		**1.18**	**0.02**
White-breasted Cormorant	**Piscivores**	**Aquatic**	**Roodeplaat Dam**	**1.57**	**0.02**
Heron sp	Piscivores	Aquatic	Soweto	2.50	0.03
Heron sp	Piscivores	Aquatic	Kempton Park	3.86	1.15
Heron sp	Piscivores	Aquatic	Parys	1.73	0.19
Heron sp	**Piscivores**	**Aquatic**		**2.50**	**0.12**
Crowned Lapwing	Omnivores/Insectivores	Terrestrial	Vanderbijlpark	0.10	0.16
Crowned Lapwing	Omnivores/Insectivores	Terrestrial	Sasolburg	0.15	0.10
Crowned Lapwing	**Omnivores/Insectivores**	**Terrestrial**	**Sasolburg**	**0.10**	**0.10**
Red-knobbed Coot	Omnivores/Insectivores	Aquatic	Soweto	1.03	14.06
Red-knobbed Coot	Omnivores/Insectivores	Aquatic	Kempton Park	3.26	0.34
Red-knobbed Coot	Omnivores/Insectivores	Aquatic	Vanderbijlpark	1.82	1.30
Red-knobbed Coot	**Omnivores/Insectivores**	**Aquatic**		**1.93**	**0.82**
Granivore	Granivore	Terrestrial	Sasolburg	0.43	2.17
Granivore	Granivore	Terrestrial	Parys	1.26	0.72
Granivore	Granivore	Terrestrial	Vanderbijlpark	0.51	12.48
Granivore	Granivore	Terrestrial	Soweto	0.50	1.07
Granivore	**Granivore**	**Terrestrial**		**0.51**	**1.28**

4.4.5. Correlations between organohalogen compound groups

Correlations quantify the degree to which two variables are associated with each other (Fowler *et al*., 2000). To assess if pollutant groups (ΣBFRs, ΣOCPs, and ΣPCBs) were related, a product-moment correlation test on log-transformed data were conducted. For a correlation to be regarded as statistically significant, it had to have a p-value smaller than 0.05 and an r-value greater than ±0.6. ΣBFRs, ΣPBDEs, HBCD was not correlated to any other pollutant groups, while ΣOCPs and ΣPCBs were correlated (p = 0.00. r = 0.85).

Correlations between ΣDDT, Σchlordanes, HCB, ΣHCHs and DL-PCBs were also investigated. The only statistically significant correlations were ΣDDT to DL-PCBs (p = 0.0 and

r = 0.81) and ΣPCBs (p = 0.0 and r = 0.84) as well as ΣHCH to DL-PCBs (p = 0.0 and r = 0.7) and ΣPCBs (p = 0.0 and r = 0.7).

4.4.6. The Ratcliffe index

The Ratcliffe index is a method to determine the eggshell thickness of bird eggs without using direct eggshell thickness measurement. The Ratcliffe index is a ratio between the egg mass, length and width, using the following equation (Dirksen *et al*., 1995):

Ratcliffe index = mass of dry shell (mg) / [length (mm) x width (mm)]

The advantage of using the index is that it allows the comparison between species. The Ratcliffe index was applied to eggshell parameters (Table S13). Since the dry mass of the eggshell was not determined, the dry mass was calculated by subtracting the contents of the eggs mass from the total mass. The width (the widest point of the egg) and length (the longest point if the egg) were not directly measured, but rather the circumference of both measurements. The width and length was calculated by dividing the individual circumferences by two. The index was not determined for the granivores because it was too difficult to obtain accurate measurements from these small and fragile shells.

The index values were then compared (one-way ANOVA) with South African data published before 1990 for the African Fish Eagle (Davies & Randall, 1989), Lanner Falcon (*Falco biarmicus*), Black Sparrow Hawk (*Accipiter melanoleucus*), Bateleur (*Terathopius melanoleucus*) (Snelling, *et al*., 1984) and Cape Vulture (*Gyps coprotherus*) (Mundy *et al*., 1982) (Supplementary data, Table S13). Species for comparison were chosen on the basis of publication availability and represented a historic comparison to the current study.

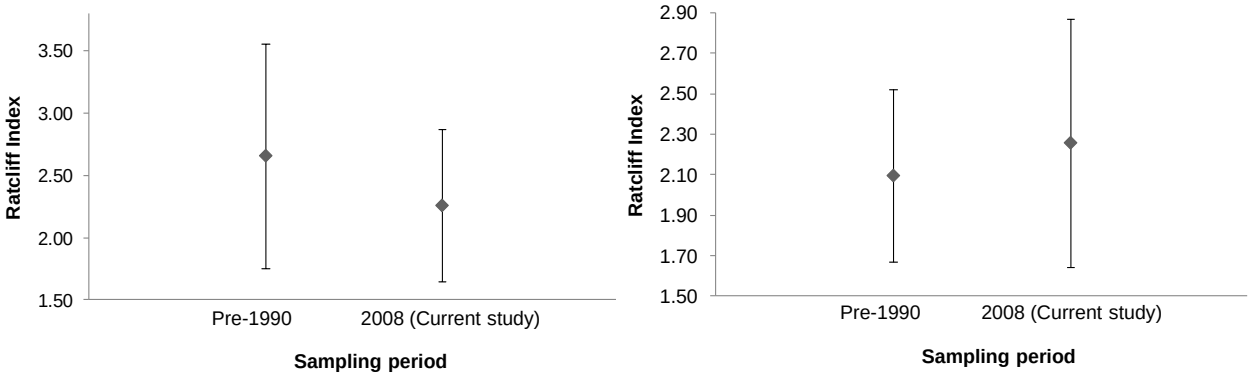


Figure 47: Comparison of historical South African data and the current study

When all species were included, the ANOVA indicated that there was no statistical differences between the current study and historical data ($p = 0.11$) (Figure 47). The data was investigated in detail and it became clear that the Cape Vulture was an outlier since it differed statistically significantly from all other species ($p < 0.05$). When this species was excluded the difference between the current study and historical data became statistically significant ($p < 0.0001$), with the current study showing an increased Ratcliffe index compared to historical data (Figure 47).

SECTION B

ORGANOHALOGEN CONTAMINANTS IN BACKYARD CHICKEN EGGS FROM THE VAAL-TRIANGLE, SOUTH-AFRICA

Twelve backyard chicken eggs were analysed from low-income residential areas in Sasolburg and Vanderbijlpark. In addition, three commercial battery eggs were analysed collected from various areas within South Africa. Levels of OCPs and BFRs were analysed as described in section 3.5 by the NVH, whereas, DLCs (2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,7,8-TCDF, 1,2,3,7,8/1,2,3,4,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8/1,2,3,4,7,9-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDF, CB-77, CB-81, CB-126 and CB-169) were determined by the Norwegian Institute for Air Research (NILU) in Norway by HR-CG/HR-MS. Due to the high costs involved in dioxin analysis, egg samples had to be pooled (Table 25). Egg parameters were determined as described in section 3.3 (Table 25).

Table 25: Pooling information and egg parameter measurements for backyard chicken eggs and commercial battery eggs from South Africa

Pools for analysis of DLCs*	Sample	Site	Eggshell thickness (mm)	Mass (g)	% Lipid	Circum-ference (cm)	Length (cm)
Pool 1	Cbr1a	Sasolburg	0.34	55	9.7	15	16
Cbr 1	Cbr1b	Sasolburg	0.34	57	8.2	15	17
	Cbr1c	Sasolburg	0.3	28	11	15	18
Pool 2	Cbr2a	Sasolburg	0.36	50	8.6	14	17
Cbr 2	Cbr2c	Sasolburg	0.31	47	13	14	16
	Cbr2c	Sasolburg	0.34	45	12	13	16
Pool 3	Tsh_a	Vanderbijlpark	0.33	51	11	14	17
Tsh	Tsh_b	Vanderbijlpark	0.3	50	11	14	17
	Tsh_c	Vanderbijlpark	0.3	49	11	14	16
Pool4	Shv_a	Vanderbijlpark	0.33	53	10	14	16
Shv	Shv_b	Vanderbijlpark	0.28	45	10	14	16
	Shv_c	Vanderbijlpark	0.34	46	10	14	16
	Bfn#	Bloemfontein	0.37	51	14	14	17
	Pta#	Pretoria	0.38	62	41	15	18
	Stb#	Stellenbosch	0.39	57	13	14	17

*Cbr: Coalbrook; Tsh: Tshepiso, Vanderbijlpark: Shv: Sharpeville, Vanderbijlpark; # commercial eggs (Bfn: Bloemfontein; Pta: Pretoria; Stb: Stellenbosch)

4.5.1. BFRs in backyard chicken eggs

Concentrations of BFRs are summarised in Table 26. In commercial eggs, only a single sample originating from Bloemfontein contained quantifiable levels of BFRs. The only PBDE congener quantified in this sample was BDE-47 (Table 26). Four of the six backyard eggs collected in Coalbrook, Sasolburg, had quantifiable ΣBFRs. Concentrations of ΣPBDE ranged between 0.1 - 0.78 ng g^{-1} wm (Table 26). All six samples collected in Vanderbijlpark area had quantifiable BFRs, with concentrations of ΣPBDEs ranging between 0.94 - 20 ng g^{-1} wm (Table 26). The congeners most frequently quantified were BDE-209 (60%), -207 (53%), and -183 (53%).

Table 26: Concentrations (ng g^{-1} wm) of PBDEs and HBCD in backyard chicken eggs

BFRs	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-206	BDE-207	BDE-208	BDE-209	ΣPBDEs	HBCD
LOD	0.02-0.05	0.04-0.05	0.02-0.05	0.02-0.05	0.02-0.05	0.02-0.05	0.04-0.05	0.1	0.05	0.02-0.05	0.05-3.12		0.2-0.29
Recovery (%)	94-103	102-105	104-117	101-106	101-105	94-104	105-106	88-112	86-113	79-115	124-230		103-131
Bfn*	ND	0.03	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.03	ND
Pta*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Stb*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cbr1a	ND	ND	ND	ND	ND	ND	0.1	ND	ND	ND	ND	0.1	ND
Cbr1b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cbr1c	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cbr2a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.14	0.14	52
Cbr2b	ND	ND	ND	ND	ND	ND	0.3	ND	0.12	0.11	0.79	1.3	2.7
Cbr2c	ND	ND	ND	ND	ND	ND	ND	ND	0.09	ND	0.69	0.78	2.7
Tsha	ND	ND	ND	ND	ND	ND	0.06	ND	0.11	ND	0.78	0.94	11.9
Tshb	ND	ND	0.08	ND	ND	ND	0.08	ND	0.19	0.09	0.79	1.2	15
Tshc	ND	ND	ND	ND	1.2	ND	3.6	0.41	0.82	0.56	2.78	9.4	0.4
Shva	ND	ND	ND	ND	0.45	ND	2.2	ND	0.24	0.1	0.67	3.7	0.43
Shvb	ND	ND	ND	ND	0.07	ND	0.33	ND	0.14	ND	0.83	1.4	11
Shvc	ND	ND	0.07	ND	1.4	ND	12	0.27	2.3	0.35	3.2	20	0.37

Note: Refer to table 25 for description of ste names; * commercial eggs

The BFR data was not distributed normally (Shapiro-Wilks test) due to the small sample size, therefore the Kruskal-Wallis ANOVA was used to determine if the concentration of BFR differed significantly between the sampling areas.

Commercial eggs had significantly lower levels of BDE-183, -209 and ΣPBDEs compared to Vanderbijlpark ($p < 0.05$), but not compared to those collected in Coalbrook ($p = 1$). Eggs from Coalbrook and Vanderbijlpark did not differ significantly from one another in the occurrence of PBDEs ($p > 0.05$). For BDE-207 eggs collected from Vanderbijlpark differed statistically significantly from both commercial eggs and those collected in Coalbrook (both, $p = 0.03$). Both ΣPBDEs (A) and HBCD (B) were more prevalent in Vanderbijlpark than in Coalbrook, Sasolburg (Figure 48).

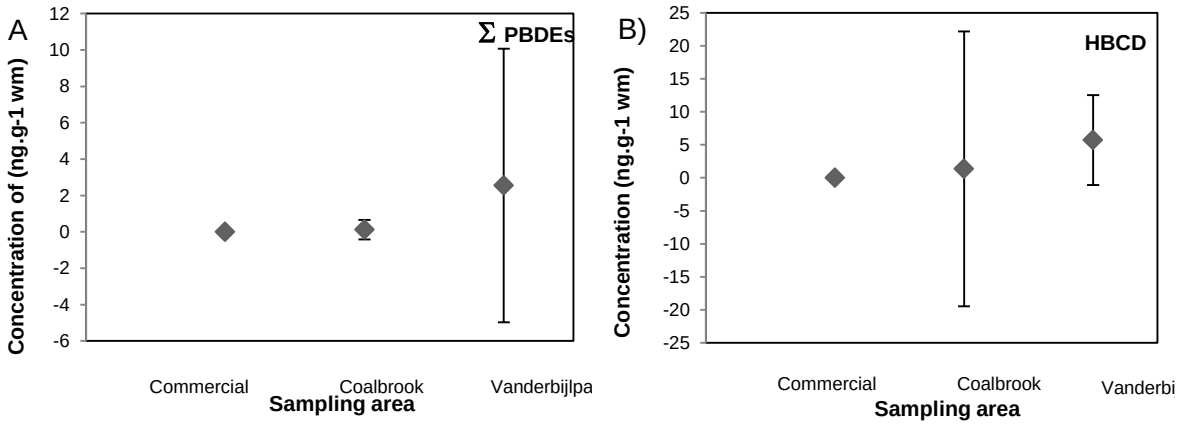


Figure 48: Median concentration of ΣPBDEs (A) and HBCD (B) in commercial and backyard chicken eggs

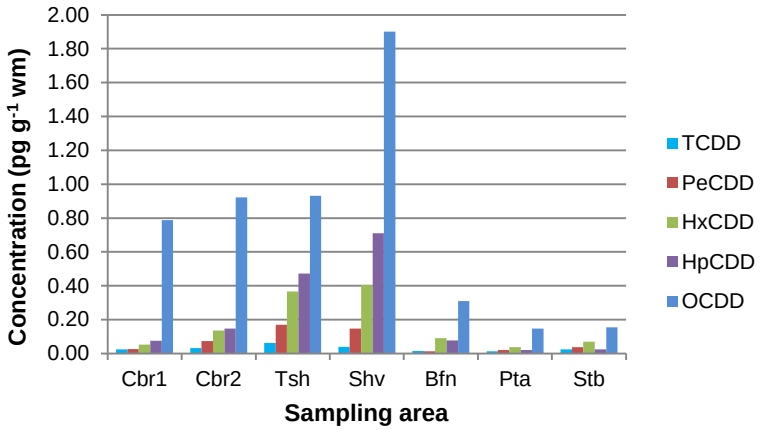
A Spearman rank order correlation showed no statistically significant correlations between BFR levels and eggshell thickness. However, BDE-183 and ΣPBDEs had a negative correlation with the length of the egg ($p < 0.05$; $r > -0.6$). HBCD and BDE-209 had a negative correlation on the circumference of the egg ($p < 0.05$; $r > -0.6$). BDE-153, -183, -206, -207, -208 and -209 were positively correlated to one another as well as ΣPBDE, with the exception of BDE-206. HBCD was positively correlated with BDE-209.

4.5.2. OCs in backyard eggs

4.5.2.1. DLCs in backyard and commercial chicken eggs

All samples had quantifiable levels of PCDD and DL-PCBs. However, PCDFs were not quantifiable in commercial eggs from Pretoria. The dominant PCDD was OCDD followed by HpCDD, with the exception of the commercial eggs where OCDD was followed by HxCDD (Figure 49). In the case of PCDFs, the dominant congener was PeCDF, with the exeption of commercial eggs from Bloemfontein where HpCDF was predominant (Figure 49) and for DL-PCBs, CB-118 (Figure 49). In all samples, there were higher mono-*ortho* PCBs compared to non-*ortho*-PCBs. For all samples DL-PCBs were the predominant DLC (Figure 50). Concentrations of DLCs in the individual eggs ranged between 76 - 5500 pg g^{-1}. The lowest concentration measured was from Coalbrook and the highest concentration from commercial eggs in Pretoria. However, if mono-*ortho's* were excluded, concentrations ranged between 0.75 – 14 pg g^{-1} wm, with the highest concentration in Vanderbijlpark.

PCDD (A)



PCDF (B)

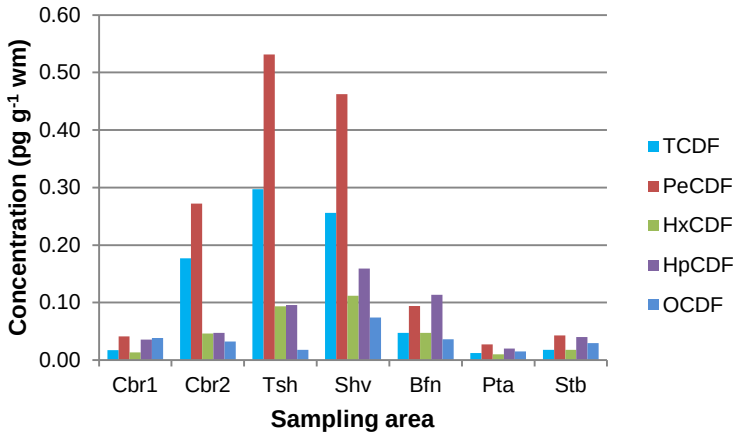


Figure 49: Concentrations of PCDD (a) and PCDF (b) congeners in chicken eggs

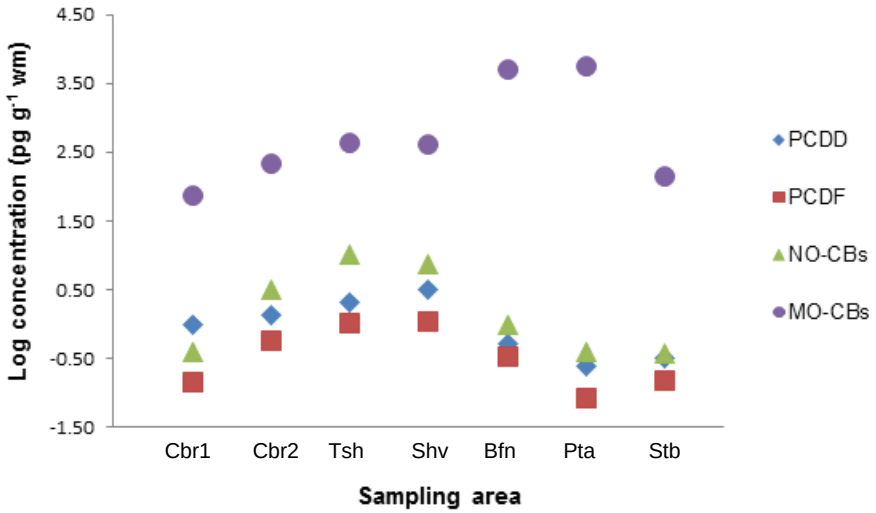


Figure 50: Comparative concentrations of DLC-groups in chicken eggs

Dioxin concentrations were converted to TEQ-values by multiplying individual concentrations with their corresponding TEF values. For these calculations, data had to be normalised to lm values. The TEQ-values (Table 27) ranged between 0.7 - 6 pgTEQ g^{-1} lm).

Table 27: The TEQ-values (pgTEQ g^{-1} lm) of DLCs found in chicken eggs from South Africa

Sample	Cbr1	Cbr2	Tsh	Shv	Bfn	Pta	Stb
2,3,7,8-TCDD	0.251	0.274	0.572	0.409	0.107	0.141	0.193
1,2,3,7,8-PeCDD	0.272	0.635	1.580	1.495	0.100	0.202	0.306
1,2,3,4,7,8-HxCDD	0.019	0.017	0.063	0.074	0.023	0.013	0.019
1,2,3,6,7,8-HxCDD	0.019	0.067	0.219	0.256	0.019	0.012	0.019
1,2,3,7,8,9-HxCDD	0.018	0.034	0.058	0.081	0.023	0.012	0.018
1,2,3,4,6,7,8-HpCDD	0.001	0.001	0.004	0.007	0.001	0.000	0.000
OCDD	0.002	0.002	0.003	0.006	0.001	0.000	0.000
2,3,7,8-TCDF	0.018	0.154	0.275	0.261	0.034	0.012	0.014
1,2,3,7,8 /1,2,3,4,8-PeCDF	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2,3,4,7,8-PeCDF	0.007	0.033	0.081	0.080	0.010	0.004	0.005
1,2,3,4,7,8/1,2,3,4,7,9-HxCDF	0.014	0.052	0.139	0.166	0.045	0.010	0.014
1,2,3,6,7,8-HxCDF	0.011	0.049	0.106	0.157	0.037	0.010	0.012
1,2,3,7,8,9-HxCDF	0.016	0.024	0.011	0.019	0.018	0.011	0.015
2,3,4,6,7,8-HxCDF	0.015	0.036	0.089	0.113	0.035	0.010	0.014
1,2,3,4,6,7,8-HpCDF	0.002	0.003	0.007	0.014	0.006	0.001	0.001
1,2,3,4,7,8,9-HpCDF	0.001	0.001	0.001	0.002	0.002	0.001	0.002
OCDF	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CB77	0.000	0.001	0.005	0.003	0.001	0.000	0.000
CB81	0.000	0.001	0.003	0.002	0.000	0.000	0.000
CB126	0.043	0.897	2.903	2.744	0.082	0.040	0.049
CB169	0.008	0.058	0.168	0.190	0.004	0.007	0.004
CB118	0.023	0.041	0.079	0.081	1.070	1.672	0.034
CB105	0.000	0.010	0.022	0.020	0.002	0.000	0.000
CB156	0.000	0.006	0.014	0.018	0.000	0.000	0.000
CB157	0.000	0.000	0.006	0.007	0.000	0.000	0.000
CB189	0.000	0.000	0.000	0.002	0.000	0.000	0.000
Total TEQ-value	**0.740**	**2.399**	**6.410**	**6.210**	**1.618**	**2.160**	**0.722**

Spearman rank order correlations were used to assess associations between individual DLCs and egg parameters. The eggshell thickness of chicken eggs was negatively correlated ($p < 0.05$; $r > -0.6$) to 2,3,7,8-TCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, CB-77, -81, -105, -156, and -157. The circumference of the egg was negatively correlated to 2,3,7,8-TCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, 2,3,7,8-TCDF, PeCDF, CB-81, -126 and -105.

4.5.2.2. OCPs in backyard and commercial chicken eggs

OCPs were quantified in all samples with ΣOCPs ranging from 0.2 – 20 ng g^{-1} wm (Table 28). HCB, β-HCH, γ-HCH and *p,p*'-DDE were found in all samples, while mirex was only quantifiable in the commercial chicken eggs from Bloemfontein (Table 28). HCB was the

predominant congener in backyard chicken eggs and ΣHCH in commercial eggs (Figure 48). β-HCH was the dominant HCH-isomer contributing more than 50% to ΣHCH in all samples, followed by γ-HCH contributing more than 20%, while α-HCH was only quantifiable in commercial eggs from Bloemfontein and Stellenbosch. ΣDDT concentrations ranged from 0.1 – 4 ng g^{-1} wm (Figure 51) with *p,p*'-DDE being the dominant compound, contributing greater than 60% to ΣDDT, followed by *p,p*'-DDT (3 – 29%) and *p,p*'-DDD (0.2 – 17%).

A Kruskal-Wallis ANOVA was used to compare sampling areas with one another. Eggs collected in Vanderbijlpark had significantly higher concentrations of HCB, oxychlordane, *trans*-nonachlor, cis-nonachlor, *p,p*'-DDE, and ΣDDT than in commercial eggs and eggs collected in Coalbrook ($p < 0.05$). β-HCH levels in commercial eggs were higher than those collected in Coalbrook ($p = 0.03$) and ΣHCH levels in Coalbrook ($p = 0.00$) were lower than both commercial chicken eggs and those collected in Vanderbijlpark ($p = 0.04$ & $p = 0.01$, respectively). For *trans*-chlordane and Σchlordanes levels differed statistically significantly between Coalbrook and Vanderbijlpark ($p = 0.03$ & $p = 0.00$ respectively) while levels of *p,p*'-DDT differed between commercial eggs and those collected in Vanderbijlpark ($p = 0.02$).

A Spearman rank order correlation showed a statistically significant negative correlation between eggshell thickness and oxychlordane, *trans*-nonachlor and *cis*-nonachlor, while the egg circumference was statistically significantly negatively correlated to HCB, oxychlordane, *p,p'*-DDT and ΣDDT.

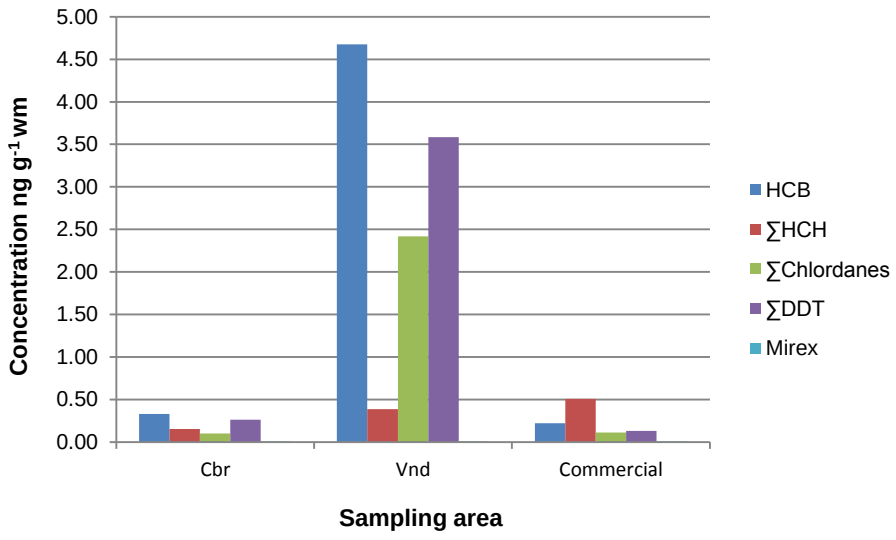


Figure 51: Concentration of OCPs in chicken eggs from the various sampling areas

Table 28: Concentrations of OCPs in chicken eggs (ng g^{-1} wm)

Sample	HCB	α-HCH	β-HCH	γ-HCH	ΣHCH	Oxychlor-dane	*Trans*-chlor-dane	*Cis*-chlor-dane	*Trans*-nona-chlor	*Cis*-nona-chlor	ΣChlor-danes	p,p'-DDE	p,p'-DDD	p,p'-DDT	ΣDDT	Mirex	ΣOCPs
Cbr1a	0.192	0.000	0.055	0.036	0.093	0.005	0.005	0.063	0.005	0.032	0.109	0.026	0.006	0.008	0.040	0.008	0.443
Cbr1b	0.079	0.000	0.022	0.016	0.040	0.005	0.005	0.026	0.005	0.016	0.057	0.016	0.006	0.008	0.030	0.008	0.214
Cbr1c	0.109	0.000	0.050	0.027	0.079	0.005	0.005	0.013	0.005	0.017	0.043	0.015	0.006	0.008	0.029	0.008	0.268
Cbr2a	0.471	0.000	0.196	0.048	0.246	0.014	0.005	0.012	0.053	0.012	0.095	0.452	0.006	0.160	0.618	0.008	1.438
Cbr2c	0.485	0.000	0.172	0.040	0.214	0.012	0.005	0.031	0.049	0.028	0.125	0.344	0.006	0.134	0.484	0.008	1.316
Cbr2c	0.524	0.000	0.212	0.034	0.248	0.018	0.005	0.023	0.048	0.036	0.130	0.388	0.006	0.153	0.546	0.008	1.457
Median	**0.332**	**0.000**	**0.113**	**0.035**	**0.154**	**0.008**	**0.005**	**0.025**	**0.026**	**0.022**	**0.102**	**0.185**	**0.006**	**0.071**	**0.262**	**0.008**	**0.879**
Stdev	**0.205**	**0.000**	**0.084**	**0.011**	**0.093**	**0.006**	**0.000**	**0.019**	**0.025**	**0.010**	**0.036**	**0.209**	**0.000**	**0.078**	**0.286**	**0.000**	**0.607**
Tsh_a	10.551	0.000	0.182	0.177	0.360	0.871	0.067	0.306	2.989	0.250	4.483	2.137	0.006	1.563	3.705	0.008	19.108
Tsh_b	8.084	0.000	1.682	0.146	1.830	1.005	0.076	0.369	3.514	0.289	5.253	2.467	0.006	1.973	4.447	0.008	19.621
Tsh_c	1.269	0.000	0.214	0.227	0.444	0.048	0.028	0.015	0.133	0.046	0.271	2.281	0.006	0.035	2.322	0.008	4.314
Shv_a	1.096	0.000	0.215	0.147	0.364	0.036	0.013	0.023	0.113	0.049	0.234	2.859	0.006	0.600	3.465	0.008	5.167
Shv_b	10.617	0.000	0.176	0.188	0.366	0.886	0.031	0.286	3.096	0.311	4.611	2.289	0.006	1.461	3.756	0.008	19.358
Shv_c	1.214	0.000	0.165	0.239	0.407	0.054	0.015	0.027	0.183	0.072	0.350	2.417	0.006	0.538	2.961	0.008	4.940
Median	**4.676**	**0.000**	**0.198**	**0.183**	**0.386**	**0.463**	**0.030**	**0.157**	**1.586**	**0.161**	**2.417**	**2.353**	**0.006**	**1.031**	**3.585**	**0.008**	**12.138**
Stdev	**4.776**	**0.000**	**0.609**	**0.039**	**0.589**	**0.481**	**0.027**	**0.166**	**1.684**	**0.127**	**2.477**	**0.249**	**0.000**	**0.745**	**0.730**	**0.000**	**7.979**
Bfn*	0.221	0.084	0.314	0.109	0.507	0.005	0.068	0.035	0.005	0.003	0.113	0.100	0.022	0.008	0.130	0.179	1.152
Pta*	0.131	0.000	0.229	0.122	0.353	0.005	0.079	0.040	0.005	0.025	0.153	0.022	0.006	0.008	0.036	0.008	0.682
Stb*	0.235	0.046	0.409	0.227	0.681	0.005	0.005	0.005	0.005	0.003	0.021	0.237	0.006	0.008	0.251	0.008	1.196
Median	**0.221**	**0.046**	**0.314**	**0.122**	**0.507**	**0.005**	**0.068**	**0.035**	**0.005**	**0.003**	**0.113**	**0.100**	**0.006**	**0.008**	**0.130**	**0.008**	**1.152**
Stdev	**0.056**	**0.042**	**0.090**	**0.065**	**0.164**	**0.000**	**0.040**	**0.019**	**0.000**	**0.013**	**0.068**	**0.109**	**0.009**	**0.000**	**0.108**	**0.099**	**0.285**

*Commercial eggs

5 Discussion

Previous studies indicated the presence of POPs in the South African environment in both abiotic and biotic matrices (Bouwman *et al*., 2008; Odusanya *et al*.,2009; Polder *et al*., 2008a; Quinn *et al*., 2008; Van Wyk *et al*., 2001). Animals inhabiting both terrestrial and aquatic environments are exposed to multiple environmental contaminants (Johnston, 1995). This can lead to interactions between toxic substances such as additive, synergistic, and antagonistic effects. Therefore, the continued monitoring and management of toxic pollutants within the environment is crucial. This chapter is divided in five sections:

- BFRs in wild bird eggs,
- OCPs in wild bird eggs,
- PCBs in wild bird eggs,
- combined occurrence of the compound classes in wild bird eggs, and
- compounds in backyard chicken eggs.

When assessing the potential toxicity, a conservative approach was used. Therefore, due to small sample sizes, single eggs, with elevated levels were seen to represent the most "sensitive" portion of the population. This was done since a conservative assessment estimates the high-end potential risk rather than the low-end potential risk (US.EPA, 2011). Additionally, due to these small sample sizes, single egg scenarios are discussed. Although no statistically meaningful data is derived therefrom, the ecological factors that can play an important role in the level of contaminants are thereby included in the discussion.

SECTION 1
BFRs IN EGGS OF WILD BIRDS WITHIN AN INDUSTRIALISED CENTRE OF SOUTH AFRICA

As discussed in chapter 2, section 2.1, PBDEs and HBCD are ubiquitous environmental pollutants due to their widespread, high volume, long-term use as flame retardants in consumer products and building material (Costa *et al*., 2008; Chen & Hale, 2010). As with the study of

Polder *et al.* (2008a), BFRs were found in all bird eggs analysed for this study, independent of sampling location or species (chapter 4, section A). Concentrations of ΣBFRs ranged between 2.6 – 43 ng g^{-1} wm, with the highest levels measured in the African Sacred Ibis and the lowest in the Crowned Lapwing (Table 19). The variation between individual species was statistically significant (one-way ANOVA, $p < 0.0001$; section 4.1.1).

The African Sacred Ibis had concentrations 1.8 times higher than any other species. This species has a unique niche compared to other avian species included in this study, which can explain the elevated levels of BFRs found in this study and by Polder *et al*. 2008a. In the absence of anthropogenic impacts, the African Sacred Ibis is a wading bird feeding mostly on insects, crustaceans, molluscs, fish, frogs, small lizards, mammals, and bird chicks (IUCN, 2010). However, the African Sacred Ibis adapts to a scavenging diet when occurring close to towns or cities. In these cases, African Sacred Ibises often feed at rubbish tips, sewage plants, abattoirs (Hockey *et al*., 2005; Polder *et al*., 2008a), and any other area rich in potential food sources. When feeding at rubbish tips, the Ibis is exposed to a wide variety of possible sources of PBDEs and HBCD including plastics, discarded electrical equipment and furniture, fabrics and polystyrene containers (Polder *et al*., 2009) as it feeds on food scraps and possibly small mammals and insects. Since most BFRs are additives mixed directly into the material they tend to leach out of the products and into environmental matrices (De Wit *et al*., 2010) from the time of production, continuing throughout the lifetime of the product including after disposal. This can then lead to increased levels of BFRs at refuge tips and consequently increased levels in animals and birds feeding there.

The high intra-species variation was most noticeable in the Red-knobbed Coot with concentrations of ΣBFRs in this species ranging between 6.07 – 208 ng g^{-1} (section 4.1.1). Congener patterns and concentrations can vary within a species due to differences in diet, overall exposure, the condition of the female bird at the time of laying, and the age of the female bird (Chen & Hall, 2010; Jaspers *et al*., 2006). However, in this dataset only one individual egg of the Red-knobbed Coot showed elevated levels (208 ng g^{-1}), while another sample collected in the same vicinity (Soweto wetland) had a concentration of 9.6 ng g^{-1}. This large value could have been caused by dietary exposure. The specific wetland in question (where African Darter eggs were also collected) does not only receive runoff from numerous industrial and municipal sources, but also from local informal solid waste dumps of both a biological and non-biological nature, including the occasional animal carcass and plastic containers. This leads to a wide range of sources of BFRs, especially to species such as Red-knobbed Coots that are

opportunistic feeders. Although they normally feed on aquatic vegetation, Red-knobbed Coots are known to feed on carrion or any other easily available food source (Hockey *et al*., 2005).

Median concentrations of HBCD ranged between 0.15 – 2.8 ng g^{-1} and for PBDEs between 0.37 – 220 ng g^{-1}, with the highest concentrations found in the African Sacred Ibis (Table 19). The congener profiles of individual BFRs can provide information on possible sources, environmental fate, and persistence of these chemicals in the environment.

5.1.1. CONGENER PROFILES OF BFRs IN EGGS

As also found by Wu *et al.* (2010), in the present study, HBCD was widely present in the environment, being detected in 72% (section 4.1.2) of the bird eggs analysed. Currently, HBCD is widely marketed as a safer alternative to PBDEs with its use and production steadily increasing (Wu *et al*., 2010). HBCD's highest concentrations were measured in Kempton Park (9 ng g^{-1}, Table S1) and Soweto (4 ng g^{-1}; Table S1). Both areas are highly populated and have various industries. HBCD is often associated with large and industrial centres (Ueno *et al*., 2006). Although HBCD has been found in the Arctic, it is believed to preverentially associate with point sources and to be one of the less mobile BFRs, indicating local sources of HBCD to the environment (Covaci *et al*., 2006).

As with HBCD, concentrations of PBDEs are also related to human population size as well as industrial activity (Henny *et al*., 2009). The highest average concentrations of PBDEs were measured in the Soweto wetlands (45 ng g^{-1}; Table S1), probably due to the same factors as discussed above.

In literature, the predominant PBDE congeners in bird's eggs are BDE-47, -99, -100, -153 and -154 (Sánchez-Prado *et al*., 2005; Costa *et al*., 2008; Gao *et al*., 2009; Chen & Hale, 2010). Among the PBDEs analysed in this study, BDE-153, -154, -183, and -47 were detected in more than 80% of samples, while BDE-28 and -209 were detected the least (Figure 17). However, PBDE congeners differed statistically significantly between species (one-way ANOVA, $p < 0.05$) with the exception of BDE-28. BDE-28 was only detected in the African Darter, although the Darter shares an aquatic diet with the White-breasted Cormorant and heron *spp*. (section 4.1.2).

As illustrated in Figure 18, the African Sacred Ibis PBDE load was dominated by BDE-209 and 183. It must be noted that BDE-47, -99, -153, -154, 183, -207, -208 and HBCD were found in 100% of the African Sacred Ibis samples, indicating a varied and wide exposure to almost all classes of PBDEs (Table 20).

The African Sacred Ibis was the only species were BDE-209 made a significant contribution to ΣPBDEs (note that this is only obvious when LOD is replaced with zero values;

Figure 18). Although deca-BDE (BDE-209) is currently the predominant PBDE-BFR produced, it was only in the African Sacred Ibis the BDE with the highest contribution. Due to the high molecular weight of BDE-209, it is assumed that uptake through the gut is limited and when it does occur that, the compound was rapidly eliminated (Sánchez-Prado *et al*., 2005). This characteristic would have made BDE-209 the least bioactive PBDE. However, as discussed in section 2.1.2, there is now evidence that certain animal species have the ability to metabolise BDE-209 through debromination to lower brominated congeners including BDE-196, -197, -206, -207, and, -208 (Van den Steen *et al*., 2007), and that BDE-209 can bio-accumulate in both terrestrial and aquatic wildlife (Jaspers *et al*., 2005; Chen & Hale, 2010).

The high levels of BDE-209 in the African Sacred Ibis (Figure 18) can be caused by two main effects. 1) Due to their unique ecology, the African Sacred Ibis has increased exposure to higher BDE-209 through point sources in rubbish tips and 2) the African Sacred Ibis cannot metabolise BDE-209.

High levels of BDE-183 may be associated with octa-BDEs in technical mixtures such as Bromkal 79-8DE or DE-79 (Guardia *et al*., 2006). Both these mixtures have high levels of BDE-183, while the congener profile of Bromkal 79-8DE is 209 > 175/183 > 207 > 197 > 203 > 206 > 196 >208 (Guardia *et al*., 2006). The congener profile for the African Sacred Ibis was 209 > 183 > 207 = 153 > 208 (Figure 18), and BDE-175, -197, -196, and -203 were not determined. This indicated exposure to an octa-BDE technical mixture that dominated other technical products as sources.

BDE-183 also dominated the PBDE profile in Heron *spp*. followed by BDE-153 and -207 (Figure 18). Since the Black-headed Heron is a terrestrial feeder (Table 13), the congener profile of heron *spp* should differ from other piscivores. The other species included in this pool were the Black-crowned Night Heron and Grey Herons, whose diet includes fish as well as small rodents, amphibians, insects and earthworms (Hockey *et al*., 2005). The presence of a terrestrial species could account for the high levels of BDE-183, which is usually associated with the terrestrial environment (Jasper *et al*., 2006).

The congener profiles of the White-breasted Cormorant and African Darter (Figure 18) were dominated by BDE-154. Although BDE-153 was a significant contributor to ΣPBDE, it was not predominant. BDE-153 is often recognised as the most persistent BDE congener (Luo *et al*., 2009a), and is often associated with birds of prey (Helgason *et al*., 2009). The congener profiles of Cattle Egrets, Crowned Lapwings, Red-knobbed Coots, Cape Sparrows, Southern Masked Weaver and Cape turtle dove (Figure 18) were dominated by either BDE-47 or -99. These two

congeners are associated with exposure to penta-BDE technical mixtures (La Guardia *et al*., 2006) or debromination of higher brominated species.

The lack of BDE-209 in the other terrestrial species does not necessarily mean that the congener is not present in the environment, since it can be metabolised with different efficiencies by different species. Variation in congener profiles of PBDE between species can be explained by differences in exposure including diet and habitat. However, NDs in the Cape Sparrows, Cape Turtle Dove, Crowned Lapwing and Cattle Egret (Table 19) indicate low levels of BDE-209 in the soil environment from this area.

5.1.2. TROPHIC LEVEL AND BFRs

Many, but by no means all, organohalogens such as BFRs biomagnify, increasing the body burdens of BFRs corresponding to trophic level. Thus, terrestrial predatory species feeding higher in the food web are expected to have higher body burdens of PBDE compared to species lower on the food web from the same region (Chen & Hale, 2010). However, care must be taken when these habitat types are compared since the exposure scenarios and diets of terrestrial and aquatic species may vary widely.

The concentration differences, in eggs of wild birds, between guilds for ΣBFRs and ΣPBDEs were statistically significant (one-way ANOVA, $p = 0.009$; section 4.1.5). However, the pattern in this study however did not correspond to an increase with trophic level: scavengers > granivores > insectivores/omnivores > piscivores (Figure 20). The scavenger, the African Sacred Ibis, was the feeding guild with the greatest levels of ΣBFRs and ΣPBDEs. The guild with the second highest concentration of ΣBFRs and ΣPBDEs was the granivores, from the lowest trophic level studied, and the lowest concentrations were found in piscivores. Piscivores are on a high trophic level within the aquatic ecosystem feeding on fish, which have the ability to bio-accumulate organohalogens both through water directly, as well as through their diet. For piscivores one of the main uptake routes is through their food (Sørmo *et al*., 2009). Therefore, higher levels are normally expected in piscivores. However, for BFRs, the exposure route is different from other major organohalogens. BFRs volatilise from consumer products, often indoors, that are then transported through particulate matter to the environment, with preferential deposition and accumulation in soil (Law *et al*., 2008). As discussed (section 3.1.1) of the granivores studied (Cape Sparrow and Cape Turtle Dove) coexist with humans since this insures supplementary food sources (Chance & Walsh, 2006) and the third species, Southern Masked Weaver is found on the outskirts of residential and industrial areas. This close association with humans, combined with the release of BFRs from human residences, likely

leads to an increased exposure to BFRs. This increased exposure is of such a nature that it exceeds the effect of trophic level and bioaccumulation in piscivores. The dominance of BDE-47 and -99 in the eggs of granivores are also the congeners most prevalent in air (Law *et al*., 2008; Figure 3), strengthening the suggested exposure route. A secondary exposure route for terrestrial animals in close association with humans is the inhalation and ingestion of dust particles that could also lead to increases in body burdens of BFRs (Law *et al*., 2008). BDE-47 and -99 were also significantly higher in granivores than in all other guilds (Figure 21).

The same argument can be made for two of the insectivore species (Cattle Egret and Crowned Lapwing). They are often found in residential areas feeding on insects in soils, leading to exposure of BFRs in soil that is very different from the piscivores. However, piscivores had statistically significantly higher concentrations of the more persistent BDE-154, -153 and 100 (Figure 21). This can once again indicate marked exposure differences between the different guilds.

The difference in congener patterns between the scavengers (African Sacred Ibis) and the granivores (Figure 21) indicates unique exposure routes for the two guilds with the highest BFR concentrations. The exposure route for the African Sacred Ibis is likely directly from BFR containing waste in rubbish tips. Granivores are likely indirectly exposed to BFRs, through the leaching of BFRs from household products into the environment.

The pattern for HBCD was different from PBDEs, in that piscivores had higher levels than insectivores and omnivores and almost equal to levels in granivores (Figure 20), indicating either different exposure routes for HBCD or differences in the uptake and metabolic efficiencies between species. There is, however, very little data available on the metabolism and uptake of HBCD in avian species.

5.1.3. FEEDING HABITAT AND BFRs IN WILD BIRD EGGS

Generally, there is a difference in the transport and fate of PBDEs between the aquatic and terrestrial ecosystems (Chen & Hale, 2010). Terrestrial birds are associated with higher brominated BFRs such as BDE-183, -201, -202, -203, -206, -207, and -209 (Jaspers *et al*., 2006; Chen *et al*., 2007). It is speculated that the higher than expected residues of nona-BDEs are due to debromination of BDE-209. BDE-209 can enter the terrestrial environment (Figure 3) through direct deposition and long-range transport, where deposition on vegetation may specifically lead to exposure to herbivores (Polder *et al*., 2009; De Wit *et al*., 2010). Aquatic birds on the other hand are associated with lower PBDE congeners, including BDE-47 (Jaspers *et al*., 2009; Luo *et al*., 2009a).

The current data indicate a statistically significant variation in PBDE concentrations between the various habitats (one-way ANOVA, $p < 0.0001$; section 4.1.6). In this study, there was a third category: combined for birds that frequently feed in both the terrestrial and aquatic habitat. Here, the combined feeding habitat (African Sacred Ibis) had higher levels of all BFRs compared to other feeding habitats, with the exception of the BDE-154, -100, -99 and -47 (Figure 22). Aquatic species had higher concentrations of BDE-154, -153, -100 and -47, with BDE-154 and -100 being statistically significantly higher than for the combined feeding habitat. Terrestrial species had higher concentrations of BDE-99 than species feeding in the combined and aquatic habitat (Figure 22).

Patterns found in the current study were different from those found in literature. Gao *et al.* (2009) found that wild aquatic birds had high levels of BDE-47, -153 and -99. Helgason *et al.* (2009) reported that birds of prey had increased levels of BDE-153. BDE-47 is generally predominant when the PBDE contamination is generalised, whereas other PBDEs can be linked to technical products (De Wit, 2002).

Aquatic bird species of this study included piscivores and the Red-knobbed Coot. The Red-knobbed Coot is an omnivore feeding mainly on aquatic vegetation, resulting in a different exposure profile than the piscivores. The terrestrial species had no representative predatory species, but consisted only of insectivores and granivores. This could affect the comparison between terrestrial and aquatic species, since these species (insectivores and granivores) are lower on the food web. Interestingly, BDE-99 that is generally found at low levels in low trophic levels in terrestrial ecosystems with higher levels found in aquatic and marine environments (De Wit, 2002) pattern was reversed in this study (Figure 22). The congener profiles that vary from literature can be due to combinations of differences in exposure profiles, metabolic capabilities of bird species tested, or other unique aspects of the life histories of South African birds. This will be discussed further in sections 5.1.5 and 5.1.6.

5.1.4. PCA ANALYSIS OF BFRs IN WILD BIRD EGGS

A PCA analysis was used to elucidate the possible influences of habitat, diet, species-specific variation, and location on the congener profiles of BFRs. On the PCA-biplot, Factor 1 (Figure 19) explained 37% of the variance. Factor 1 was a contrast between the higher-brominated PBDE congeners (BDE-207, -208, -183, and 153) with negative scores and the lower-brominated PBDE congeners (BDE-47, -100, -28 and higher brominated BDE-154) with positive scores. In the PCA-biplot the African Sacred Ibis and Heron *spp* associated with the higher brominated PBDEs, while the African Darter and White-breasted Cormorants associated

with the lower brominated PBDEs and BDE-154. This pattern appeared to be influenced by the diet of these birds. Although the Heron *spp*. were grouped with the piscivores, both the African Sacred Ibis and Heron *spp* are opportunistic feeders that will utilise the terrestrial habitat for food. This could account for the strong association on the PCA-biplot between BDE-183 (a congener associated with terrestrial habitats as discussed earlier), and nona-BDE congeners that are associated with the degradation of BDE-209. In general, terrestrial sources are the main contributor to BDE-209 in the environment. Therefore, BDE-209 is normally prevalent in the terrestrial food web (Luo *et al*., 2009a). The non-significant association of terrestrial granivores and insectivores with BDE-209 could be due to their lower position in the food web, the strong association of BDE-209 with soil particles, and the differences in their metabolic and/or retention efficiencies of higher brominated PBDE congeners. As was also shown in literature (Jaspers *et al*., 2006; Luo *et al*., 2009a), the piscivores (African Darter and White-breasted Cormorant) and the aquatic Red-knobbed Coot were associated with BDE-47 on the PCA-biplot, as commonly found in aquatic species.

Factor 2 on the PCA-biplot (Figure 19) explained 32% of the variance within the dataset and was mainly a contrast between BDE-99 with positive scores, and BDE-153 with negative scores. This separated the species on the PCA-biplot into two broad groupings: species on a lower trophic level with positive loadings, and species on a higher trophic level with negative loadings. This agrees with patterns in literature where predators (high on the trophic level) are associated with BDE-153 (Helgason *et al*., 2009). Although BDE-99 is often associated with piscivores, this biplot indicated an association with low trophic levels, with an exception of two African Darters. However, these samples were of the few that contained BDE-28. This could have affected their placement in the PCA-biplot. BDE-99 is one of the most important congeners detected in air samples as discussed in section 5.1.2. This could lead to increased exposures of granivores, insectivores, and herbivores through dust and particle deposition from air. Although classed as an omnivore, the Red-knobbed Coot mainly feeds on aquatic plants. As with other plants, air deposition can lead to increased concentrations of BDE-99.

HBCD, BDE-209 and BDE-206 were located close to the centre of the PCA-biplot, indicating that they did not significantly contribute to either factor 1 or 2. There was also no clear grouping according to sampling location in the PCA-biplot.

5.1.5. BFR-CONGENERS AND EGGSHELL THICKNESS

Correlations are used to determine the degree to which two or more factors are related to one another statistically. The p-value will indicate the reliability of the correlation and r will

indicate the extent to which the values are proportional and if the correlation is linear. As discussed earlier in the case of POPs, positive correlations between congeners of a pollutant group can indicate similar routes of exposure and/or mechanisms of accumulation (Stapleton & Baker, 2003).

The individual PBDEs did not show strong correlations to one another. HBCD was significantly correlated to BDE-153, -183 and -207. Strong significant linear correlations do not only indicate similar sources, but also suggest debromination leading to similar congener profiles (Sudaryanto *et al*., 2008). However, it is hard to make any conclusion of this kind from the current data. It can, however, be surmised that correlations of high-brominated PBDEs with HBCD may indicate the continued use of deca-BDE technical product. As discussed in section 5.1.1., BDE-209 can be debrominated to lower chlorinated species, indicating the release of deca-209 together with HBCD.

Levels of organohalogen compounds are generally linked to negative impacts on eggshell thickness (Fernie *et al*., 2009) that can consequently lead to increase egg breakage during egg incubation (as discussed in section 2, Chapter 2). Only granivore species showed any negative correlation between PBDEs and eggshell thickness. Relevant congeners were BDE-100, -153, -183, and -207. As discussed by Bouwman *et al*. (2008), an eggshell reduction of >18% is associated with population declines. However, this benchmark was not used in the current scenario. The granivore guild consists of three distinct species, with six samples analysed in total. This small sample size excludes less robust statistical analysis such as linear regression. Additionally, no historical data is available for comparison. Therefore, there were no reference values to benchmark eggshell thinning against current levels.

5.1.6. COMPARISONS OF BFRs FOUND IN WILD BIRD EGGS

It is difficult to compare data between published studies, since they comprise a wide variety of species, tissues analysed, the number of congeners analysed (Luo *et al*., 2009a), and the anthropogenic impact of a given study area. There is an increasing trend to report concentration on an lm-basis, facilitating the comparison between species. Therefore, for all comparisons to published literature, concentrations were converted to lm. PBDEs can degrade through the effects of photolytic degradation and metrological characteristics can be of importance to understand their fate in abiotic matrices and thus the levels in the food web. Photolytic degradation initially leads to debromination of higher brominated species with degradation kinetics correlated to the number of bromines, as well as the stereo-configuration of the parent molecule (Fang *et al*., 2008). This is particularly true for studies conducted in

southern Africa. South Africa has a climate with relatively low rainfall, high levels of sunlight, and a temperate climate. Since this can affect the congener pattern and concentration of BFRs, levels measured in the environment might not correlate to those being released.

Levels of HBCD and PBDEs found in selected literature and from the current study is summarised in Table 29. Levels measured in piscivore species during this study were approximately 38 times lower than the levels measured in the United States of America (USA) (Park *et al*., 2009) and in the Great Lakes of Canada (Gauthiet *et al*., 2009, Table 29). However, the levels from this study are in the same range as in marine piscivorous birds from the Antarctic and St.Lawrence Canada (Yogui & Sericono *et al*., 2009; Lavoie *et al*., 2010, Table 29). PBDEs were and are currently produced in the USA. The area where the USA studies were conducted had specific point sources of organic contamination (Henny *et al*., 2009). Therefore, high levels of POPs were expected in these birds. The concentrations measured in the Canadian Herring Gulls come from diverse areas including harbours and industrially impacted sites that could also lead to increased levels of contamination. Although the Herring Gull is often seen as a scavenger, it was considered a facultative piscivore (Gauthier *et al*., 2008).

Levels of HBCD could not be compared as comprehensively since this compound had rarely been analysed. When analysed, levels from other studies were on average 10 times lower than levels measured in the current study (Table 29). Levels of ΣPBDEs and HBCD in the African Darter in the current study are 11 times higher that those measured in Parys, South Africa by Polder *et al*, (2008a). This could be due to the differences in sampling locations.

The African Darters collected during this study were predominantly from Kempton Park, an area highly impacted by anthropogenic changes. The dam where these eggs were collected was drying out at the time of collection, possibly causing changes in the sediment, fish population dynamics, and eventually the condition of the birds. Changes to a system's natural flow correspond to changes in the aquatic ecosystem. This was evident in the sampled dam where there was an increase in water vegetation, to an extent, where almost the entire water surface was obscured. Drastic changes to the plant community will cause changes to the water chemistry, including the pH (Tucker & D'Abramo, 2008). Sediment is recognised as one of the main sinks for organic pollutants (Zounis *et al*., 2001). However, these pollutants can be remobilised through changes in the water chemistry such as pH and redox potential (Zounis *et al*., 2001; Arle, 2002; Bowman *et al*., 2002). This in turn can lead to increased levels in the water body (Zounis *et al*., 2001) that will directly affect the exposure of fish to POPs.

Table 29: PBDE and HBCD concentrations in the current study and others

Reference	Country	Species	Scientific name	Year	Area activity	Feeding	n	Compound (ng g^{-1}) lm									
								BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-209	HBCD	∑PBDEs
Polder *et al.*, 2008a	RSA	African Darter	*Anhinga rufa*	2004-2005	Industrial	Piscivore	14	0.17	1.2	0.2	3	5.2	6.6	1.1	0.13	3	17
		Reed Cormorant	*Phalacrocorax africanus*	2004-2005	Industrial	Piscivore	3	0.23	1.8	1	2.3	1.8	4.7	2.2	0.17	2.3	14
		Cattle Egret	*Bubulcus Ibis*	2004-2005	Reserve	Insectivore	11	0.14	0.14	0.1	0.21	0.2	0.04	1.9	0.11	1.4	2.3
		Cattle Egret	*Bubulcus Ibis*	2004-2005	Industrial	Insectivore	9	0.17	0.03	0.5	0.2	0.6	0.5	1.7	0.2	1.72	3.7
		African Sacred Ibis	*Threskiornis aethiopicus*	2004-2005	Industrial	Scavenger	2	0.17	6.2	28	12	40	12	110	18	38	230
		Crowned Plover	*Vanellus coronatus*	2004-2005	Agriculture	Insectivore	1	0.1	2.9	1.6	0.3	21	3.1	89	2.7	1.6	120
		Little Grebe	*Tachybaptus ruficollis*	2004-2005	Agriculture	Piscivore	1	0.21	8.3	7.2	1.3	1.1	1	0.42	0.16	2.1	19
		White-fronted Plover	*Charadrius marginatus*	2004-2005	Coastal-industrial	Insectivore	1	0.09	2.6	2.2	0.5	0.5	0.6	0.6	0.06	0.91	7.1
		Kelp Gull	*Larus dominicanus*	2004-2005	Coastal-industrial	Scavenger	1	0.1	5.3	1.3	1.4	0.1	1.1	0.4	0.07	1	9.4
Jaspers *et al.*, 2005	Belgium	Little Owl	*Athene noctua*	1998-2000	Urban	Terrestrial predator	39	1	36	56	6	32	6	12	NA	NA	150
Fängström *et al.*, 2005	Faroe Island	Fulmar	*Fulmarus glacialis*	2000-2001	Marine	Piscivore	16		4.3	4.6	1.6	5	5		0.27	NA	21
Henny *et al.*, 2009	USA(Seattle)	Osprey[a]	*Pandion haliaetus*	2006-2007	Industrial	Piscivore	11	98	3700	910	1050	390	440	0.5	0.5	0.5	7300
	USA(Columbia River)	Osprey[a]	*Pandion haliaetus*	2006-2007	Industrial	Piscivore	20	130	8500	470	2800	710	600	0.95	0.5	0.5	13500
She *et al.*, 2008	USA(San Francisco)	Caspian Tern	*Sterna caspia*	2000-2003	Industrial	Piscivore	58		2700	1003	810	303	700	NA	NA	NA	5100
		Foster's Tern	*Sterna forsteri*	2000-2003	Industrial	Piscivore	76		3400	1450	600	404	250	NA	NA	NA	6200
		Least Tern[b]	*Sterna antillarum browni*	2000-2003	Industrial	Piscivore	10		2500	1700	1000	205	190	NA	NA	NA	5700
		Clapper Rail[b]	*Rallus longirostris*	2000-2003	Industrial	Omnivore	4		160	103	78	38	17	NA	NA	NA	400
Dauwe *et al.*, 2009	Belgium	Great Tit[c]	*Parus major*	2006	Industrial	Insectivore	15		15	16	4	12	1.6	3.5	NA	NA	49
		Northern Lapwing[c]	*Vanellus vanellus*	2006	Industrial	Insectivore	13		33	34	18	15	62	4	NA	NA	109
		Mediterranean Gull[c]	*Larus melano-cephalus*	2006	Industrial	Insectivore	6		8.5	5.2	1.3	4.1	0.5	2.7	NA	NA	25
Helgason *et al.*, 2009	Norway	Herring Gull	*Larus argentatus*	2003	Marine	Scavenger	10	7	410	450	70	19	16	NA	NA	NA	570
		Black-legged Kittiwake	*Rissa tridactyla*	2003	Marine	Piscivore	10	7	140	16	16	10	7	NA	NA	NA	200
		Atlantic Puffin	*Fratercula arctica*	2003	Marine	Piscivore	10	4	41	21	11	6	9	NA	NA	NA	90

Table 29 continued: PBDE and HBCD concentrations in the current study and others

Reference	Country	Species	Scientific name	Year	Area activity	Feeding	n	Compound (ng g^{-1}) lm									
								BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-209	HBCD	∑PBDEs
Yogui & Sericono *et al*., 2009	King George Island (Antarctic)	Chinstrap Penguin	*Pygoscelis antarctica*	2004-2005	Marine	Krill	35	NA	2.6	2.6	0.7	NA	NA	NA	NA	NA	6.8
		Gentoo Penguin	*Pygoscelis papua*	2004-2005	Marine	Krill	17	NA	2.9	3.4	0.8	NA	NA	NA	NA	NA	8.1
		South Polar Skua	*Catharacta maccormicki*	2004-2005	Marine	Piscivore	13	NA	53	14	29	NA	NA	NA	NA	NA	150
Lavoie *et al*., 2010	Gulf of St. Lawrence Canada	Great Black-backed Gull[a]	*Larus marinus*	2006-2007	Marine	Scavenger	18-22	NA	830	380	200	150	72	NA	NA	93	1600
		Herring Gull[a]	*Larus argentatus*	2006-2007	Marine	Scavenger	18-22	NA	1400	1700	360	220	110	NA	NA	120	3800
		Black-legged Kittiwake[a]	*Rissa tridactyla*	2006-2007	Marine	Piscivore	18-22	NA	280	140	470	36	18	NA	NA	53	520
		Common Eider[a]	*Somateria mollissima*	2006-2007	Marine	Omnivore	18-22	NA	110	11	6.7	3.6	2.6	NA	NA	15	130
		Razorbill[a]	*Alca torda*	2006-2007	Marine	Krill	18-22	NA	930	360	290	66	72	NA	NA	101	1700
		Black Guillemot[a]	*Cepphus grylle*	2006-2007	Marine	Piscivore	18-22	NA	420	130	100	28	35	NA	NA	6	720
Park *et al*., 2009	USA, California	Peregrine Falcon[d=15]	*Falco peregrinus*	2000-2007	Mixed	Terrestrial predator	12	NA	550	4000	1400	19000	2060	4000	2200	NA	467000
Gauthier *et al*., 2008	Canada, Laurentian Lakes	Herring Gull[d=39]	*Larus argentatus*	2005-2006	Industrial	Piscivore	70-91	30	1800	2900	1200	700	570	75	130	NA	7800
Linberg *et al*., 2004	Southern Sweden	Peregrine Falcon	*Falco peregrinus*	1992-1999	Mixed	Terrestrial predator	30	NA	270	1090	450	1300	240	310	130	99	3800
Current study	RSA	African Sacred Ibis	*Threskiornis aethiopicus*	2008-2009	Mixed	Scavenger	16	0.17	3.9	13	4.9	120	12	320	270	46	870
		African Darter	*Anhinga rufa*	2008-2009	Mixed	Piscivore	13	0.67	25	33	27	11	32	5.04	37	36	180
		White-Breasted Cormorants	*Phalacrocorax carbo*	2008-2009	Mixed	Piscivore	4	0.24	20	1.8	17	23	31	2.5	47	15	150
		Heron *spp*.		2008-2009	Mixed	Piscivore	6	0.16	0.66	2.9	1.6	87	3.8	106	66	4.3	280
		Cattle Egret	*Bubulcus Ibis*	2008-2009	Mixed	Insectivore	6	0.15	2.3	42	1.2	4.8	4.5	3.8	23	4.6	84
		Crowned Lapwing	*Vanellus coronatus*	2008-2009	Mixed	Insectivore	6	0.09	5.9	5.5	1.4	2.99	1.2	3.3	14	1.9	40
		Red-knobbed Coot	*Fulica cristata*	2008-2009	Mixed	Omnivore	6	0.08	11	39	1.3	1.04	0.64	0.49	250	5.7	315
		Cape Turtle Dove	*Streptopelia capicola*	2008-2009	Mixed	Granivore	1	0.12	0.23	130	0.39	0.89	0.78	0.23	18	4	150
		Cape Sparrow	*Passer melanurus*	2008-2009	Mixed	Granivore	3	0.17	180	180	0.17	2.6	1.00	2.6	27	6.40	400
		Southern Masked Weaver	*Ploceus velatus*	2008-2009	Mixed	Granivore	4	0.22	890	150	2	17	9.08	13	48	13	1100

NA: Not analysed; a: BDE154/BB153; b: Failed to hatch; c: Median concentrations; dx: ∑PBDEs more than shown x = nr of congeners;

These changes will eventually lead to changes in the population of the dam. Fish will eventually die due to changes in pH, low dissolved oxygen content and increased dissolved solids. These fish are then consumed by bird species, leading to an increased exposure to organic toxicants in birds.It must also be taken into account that birds are mobile and can use food sources relatively distant from their nesting areas. The entire Gauteng is industrialised with a high population density, most likely increasing exposure of birds to organic pollutants. The increased levels of BFRs in the current study compared to the findings of Polder *et al*, (2008a), can also suggest an increased input of BFRs over time (increasing time-trend). Thus instead of environmental levels decreasing with time, as it does in Europe, following increased regulations (Sjödin *et al*., 2004), we are seeing increased environmental levels with time. This could be caused by the import of non-European products from countries where such regulation has not yet come into force. This increased concentration time trend warrants further research into the occurrence of BFRs in the South African environment.

In the case of scavengers, marine scavengers consisting of Gull *spp*. had comparable levels to those found in the African Sacred Ibis in the current study (Helgason *et al*., 2009; Lavoie *et al*., 2010). With exception of the Herring Gulls collected in gulf of St. Lawrence Canada (Lavoie *et al*., 2010), these gulls had PBDE levels four times higher than the levels measured in the African Sacred Ibis. The African Sacred Ibis from the current study had levels almost four times higher than reported by Polder *et al*. (2008a). This difference could be due to the larger sample size (n = 13) since previous levels were reported for only two individual eggs. Additionally, Polder *et al*, (2008a) reported on eggs from Parys, which is much less industrialised compared to the Soweto wetlands where the current samples were collected (see section 3.2).

The highest concentration of lipid-corrected ΣPBDEs were found in the Southern Masked Weaver (1100 ng g^{-1} lm) that was comparable to concentrations measured in the Great Black-backed Gull from the Arctic (Lavoie *et al*., 2010), the Razorbill from the Canadian marine environment (Lavoie *et al*., 2010), and the African Sacred Ibis in the current study. This high level in the Southern Masked Weaver that is on an otherwise low trophic level is surprising. This finding can, however, be ascribed to its close association with humans as described in section 5.1.2. From the literature survey (Table 29), the highest concentrations were measured in the Peregrine Falcon, California, USA (Park *et al*., 2009). These levels were 35 times higher than measured in the Osprey in Seattle and San Francisco, California (Henny *et al*., 2009). This could therefore indicate large differences between terrestrial and aquatic exposure profiles to PBDEs and other BFRs. However, such conclusions are speculative only, since there are

exposure differences, species-specific variations in the bioaccumulation and biotransformation of PBDEs, and the differences in trophic level. More targeted research is needed to elucidate this finding, as many terrestrial birds would be similarly exposed in many industrialised areas in Africa.

5.1.7. POTENTIAL TOXICITY ASSESSMENT OF BFRs IN WILD BIRDS

Evidence from laboratory studies suggests that PBDEs have wide-ranging negative impacts on bird populations (McDonald, 2002; Fernie *et al*., 2005; Fernie *et al*., 2006; Chen & Hale, 2010). However, to date there are no fixed regulatory or toxicological levels to benchmark PBDE and HBCD contamination in birds eggs. From literature, the following was summarised (Fernie *et al*., 2005; Fernie *et al*., 2006; Henny *et al*., 2009; Chen & Hale, 2010):

- In ova, levels of 18.7 μg g^{-1} wm ΣPBDEs per egg have been linked to changes in hormone homeostasis, increased oxidative stress, and immunological changes in the American Kestrel (*Falco sparverius*).
- Physiological changes associated with high concentrations of PBDEs include increased growth, weight gain, increased food consumption, bone length increase, feather length disrupting, and normal energetic homeostasis in nestlings.
- Levels in American Kestrel and chicken eggs exceeding 1.8 to 3.2 μg g^{-1} wm per egg caused decreased hatching success and increased sub-lethal effects.
- For the Osprey, a no observed effect level (NOEL) of 1 μg g^{-1} wm has been reported.
- High levels of PBDEs in adult birds have been linked to delayed egg laying, smaller eggs, reduced eggshell thickness, reduced fertility, and reduced reproductive success.

None of the eggs analysed for this study had concentrations of ΣPBDEs approaching the published effects levels in birds. However, no strong conclusions on safety can be drawn from this, since the toxicological effects of PBDEs have never been studied on any of the present species under South African conditions. South African birds have exposure profiles different from elsewhere. Many species, or their trophic and taxonomic equivalents, that are migratory in the Palaearctic or Northern Africa are non-migratory in South Africa (Hockey *et al*., 2005; Birdlife International, 2010). Migratory behaviour will not affect or even reduce exposure as would possibly occur with migration to less polluted areas. Therefore, non-migratory birds will presumably experience almost constant exposure levels (although mediated by seasonality and other factors) as found in the South African environment.

Furthermore, there are differences in life history characteristics of birds in the southern hemisphere when compared to the northern hemisphere equivalents, as discussed in section 2.5. Smaller clutch sizes combined with longer developmental periods and increased longevity could lead to increased levels of POPs in adult birds compared to other northern populations. Additionally for PBDEs, concentrations measured in the tissue of female birds are higher than those found in eggs (Verreault *et al*., 2006; Van den Steen *et al*., 2009). However, there is disagreement in literature as to whether maternal transfer to eggs promotes more persistent hydrophobic (Van den Steen *et al*., 2009), or less persistent PBDE congeners (Verreault *et al*., 2006), depending on the species studied. This variation in results concerning the maternal transfer of PBDEs, could be species-specific or influenced by diet and exposure profiles.

The fact remains that adult birds have higher levels of PBDEs, than those measured in their eggs. Therefore, combined with the unique southern hemisphere life history characteristics, the toxicological impacts and risk from BFRs could be more severe in adult birds than in eggs.

These findings indicate the requirement for further research into the occurrence of BFRs in wild birds of South Africa. The use of adult bird tissue in conjunction with eggs should be investigated to better understand the risk to bird populations as a whole, since negatively impacted adult birds can result in reduced reproductive success and eventually impact on bird populations.

SECTION 2

OCPs IN THE EGGS OF WILD BIRD POPULATIONS WITHIN INDUSTRIALISED CENTRES OF SOUTH AFRICA

Although few of the historic OCPs are currently in use, it is important to determine and understand the implications of the levels of OCPs and their main metabolites in the environment due to their bio-accumulative potential and chronic adverse health effects in both humans and the environment (Jaspers *et al*., 2005). Although the majority of OCPs have been banned since the 1970s, concentrations in the environment remain high due to the inherent recalcitrant nature of these compounds, combined with atmospheric transport from areas where these compounds are actively used and/or produced. As in the case of DDT, although DDT was deregistered for agricultural use in 1976 in South Africa, it is still used, as mandated by Annex B, part II of the SC, in the northeastern areas of South Africa to combat malaria (Stockholm convention on persistent organic pollutants, 2009).

All eggs had detectable levels of OCPs with the median concentration ranging from 4.2 – 620 ng g^{-1} with the highest median levels found in the White-breasted Cormorant, the top predator studied (Table 21). The lowest levels were in the Cape Turtle Dove, a granivore (Table 21). The concentrations of OCPs were significantly different between species (one-way ANOVA $p < 0.0001$, section 4.2.1). The highest single concentration measured was in an individual African Darter egg collected in Kempton Park (2 600 ng g^{-1}, *p,p*'-DDE contributing 2 400 ng g^{-1}, Table S4).

As discussed in section 3.2, although relatively little literature has been published on the use and production of OCPs in South Africa, it is known that lindane and DDT were produced in Kempton Park (Osibanjo *et al.,* 2002). Additionally, the dam in Kempton Park where African Darter and Red-knobbed Coot eggs were collected was almost dry (as discussed in section 3.2 and 5.1.5) placing stress on organisms dependent on the dam for food, water, and shelter. The changes in the chemical and physical environment can cause trapped OCPs to become bioavailable (Zounis *et al*., 2001; Bowman *et al*., 2002). These factors, in turn, can lead to increased levels of organohalogenated compounds in birds frequenting the impacted area. The mean level of ΣOCPs in the bird eggs of wild bird species in declining order was White-breasted Cormorant > African Darter > Heron > Red-knobbed Coot > African Sacred Ibis > Southern Masked Weaver > Cattle Egret > Crowned Lapwing > Cape Turtle Dove > Cape Sparrow (Table 21).

HCB, β-HCH, oxychlordane, *cis*-nonachlor, *p,p*'-DDT, *p,p*'-DDD, and *p,p*'-DDE had concentrations above the quantification limit in all samples (section 4.2.1). Although mirex was never registered for use as an insecticide, it was found in 72% of samples, the lowest detection frequency for all OCPs. Mirex is extremely persistent and capable of undergoing long-range transport (Braune, 2007), thus contaminating areas were it was never actively used. That mirex was ubiquitously present in low levels in all eggs suggests background concentrations (Guruge *et al*., 1997; Bouwman *et al*., 2008). However, there was one sample (Heron *spp*., 38 ng g^{-1}) with elevated levels of mirex. Another possible explanation for the occurrence of mirex in the South African environment is its use as a flame retardant in numerous commercial applications (Bouwman *et al*., 2008). Nothing is known about this route of entry and its subsequent release in South Africa. Since levels of mirex did not differ significantly between species (one-way ANOVA, $p > 0.05$, section 4.1.2), the likely source was through long-range transport.

5.2.1. CONGENER PROFILES OF OCPs IN WILD BIRD EGGS

To compensate for the large variations in concentration levels between species, the mean percentage distribution for OCPs were calculated for each species and their patterns compared (Herzke *et al*., 2002). Composition profiles for OCPs in all species, excluding the Crowned Lapwing and Cape Sparrow, were dominated by the DDT group, followed by HCB and ΣHCH (Figure 24). The predominance of DDT and its metabolite is the pattern generally reported in literature (Guruge *et al*., 1997; Jaspers *et al*., 2005; Braune, 2007; Luo *et al*., 2009a).

The relatively high levels of HCB are expected due to current unintentional production during industrial processes and incomplete combustion (Bailey, 2001). Additionally, HCB has a long halflife, with degradation taking as long as 6 years, depending on the climatic conditions of the region (Barber *et al*., 2005). The highest concentration of HCB was measured in Crowned Lapwings collected in Vanderbijlpark and Sasolburg (Table S4). Vanderbijlpark's industries are dominated by steel production and steel processing while Sasolburg's main industry is petrochemical manufacturing, both known sources of HCB (Bailey 2001; Barber *et al*., 2005; Murakami *et al*., 2008). The low levels of lindane (γ-HCH), that is currently applied in South Africa on agricultural crops such as maize, wheat, cotton and sunflowers (Nel *et al*., 2002; Batterman *et al*., 2008), was unexpected.

The composition profile for DDT (Figure 25) was similar among all species. For the majority of species, as reported in literature, *p,p'*-DDE was the dominant metabolite contributing more than 90% to ΣDDT (Guruge *et al*., 1997; Jaspers *et al*., 2005; Braune, 2007; Luo *et al*., 2009a). DDE is more persistent than either the parent compound DDT or the metabolite DDD

(de Cruz *et al*., 1997), and is generally the predominant metabolite of DDT when contamination is contributed to historic use, or transported from distant applications.

As found by Bouwman *et al*. (2008), there were quantifiable levels of *p,p*'-DDT in all eggs analysed, with concentrations ranging between 0.1 – 7.2 ng g^{-1} wm (Tabel 22; Figure 25). This is a rare occurrence since *p,p*'-DDT's presence in biological samples is usually associated with current use of technical DDT. As described by Bouwman *et al*. (2008), the presence of *p,p*'-DDT can indicate atmospheric transport from the north-eastern areas of South Africa where technical DDT is still used, illegal usage, or leakage from old stores. However, the fact that levels within the various species did not differ statistically significantly (one-way ANOVA, $p > 0.05$, section 4.2.1) and that DDT levels were comparable indicated that the contamination is likely associated with long-range or atmospheric transport, rather than specific point sources.

The congener profile for HCH (Figure 26) indicated that β-HCH was the dominant congener in all species except for the Southern Masked Weaver, Cape Turtle Dove, and Cape Sparrow, which are the granivores, the lowest trophic level studied here. Their diet consists mainly of seeds where the high contribution of γ-HCH may be explained by its use as a seed coating in agriculture or its use in domestic gardens. The presence of γ-HCH is associated with current use since this isomer degrades more rapidly than α-HCH and β-HCH (Wu *et al*., 1997). β-HCH, on the other hand, is associated with historic use since it is normally present at low concentrations in lindane and technical HCH products (Guruge *et al*., 1997). β-HCH can be formed during the isomerisation of α-HCH (Wu *et al*., 1997) and the degradation of lindane (Wang *et al*., 2007). Furthermore, β-HCH is more resistant to metabolic degradation and bio-accumulates in biota (Konstantinou *et al*., 2000).

Oxychlordane was the predominant chlordane constituent, contributing between 17-83%. However, levels of *trans*-chlordane were also noticeable, contributing 6-74% to Σchlordanes (Figure 27). Levels of the various chlordanes did not differ significantly from one another except for significantly lower concentrations of oxychlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, and Σchlordanes in the Cattle Egret (one-way ANOVA; $p < 0.05$, section 4.2.1). Although chlordane has been deregistered in South Africa, existing stockpiles are used for termite control (Bouwman *et al*., 2008). High levels of chlordane and nonachlor congeners (Figure 27) indicate current use since technical chlordane consists of a mixture of structurally related compounds with the major constituents *trans*-chlordane, *trans*-nonachlor, *cis*-chlordane and *cis*-nonachlor (Dearth & Hites, 1991). When released into the environment, these compounds are metabolised to a number of epoxide metabolites of which oxychlordane is prevalent (Bondy *et al*., 2003). Another explanation for this could be retention of parent-

chlordane related compounds in soil, years after initial application. This factor could be significant since stockpiles should be close to-if not depleted.

Species dependent differences in the composition of OCPs have been recorded in other studies (Herzke *et al*., 2002). The Crowned Lapwing had a unique OCP profile when compared to all other bird species analysed: Σchlordanes > HCB > ΣDDT > ΣHCH > mirex (Figure 24). The predominance of chlordanes over DDT in Lapwings has been reported in another study by Dauwe *et al*. (2009). At first glance, it would be easy to attribute high levels of Σchlordanes in the Crowned Lapwing to its diet and thus exposure profiles since termites are their major food source and chlordane is used in termite control. This exposure route is important when looking at the increased concentration of chlordane in the Crowned Lapwing compared to other species. However, the similar results of Dauwe *et al.* (2009), working on the Northern Lapwing (*Vanellus vanellus*) from the harbour area in Antwerp indicates that the metabolic efficiency of the Northern Lapwing for OCs might be different when compared to other avian species.

Dauwe *et al*. (2009) postulated that the Northern Lapwing might be able to metabolise DDE more efficiently, leading to a different pollutant ratio for this species. Current results lend validity to this hypothesis. However, no biochemical research on the metabolic efficiencies of Lapwings could be found in literature. Results from the previous South African study (Bouwman *et al*., 2008) could not be used in this comparison, due to the small number of Lapwing samples collected (single eggs from only two species) and the low levels of pollutants found (ND for Σchlordanes). The Σchlordane results in the Crowned Lapwing of the present study once again indicate the importance of diet and metabolic efficiencies in the differential exposure profiles of different bird species.

5.2.2. INFLUENCE OF TROPHIC LEVEL ON THE OCCURRENCE OF OCPs IN WILD BIRD EGGS

Dietary intake of OCPs is the most important factor in the accumulation of these compounds within an organism. Therefore, higher levels of OCPs are expected at higher trophic levels in both the aquatic and terrestrial environment (Guruge *et al*., 1997).

The one-way ANOVA showed a statistically significant ($p < 0.0001$) difference in the concentration of OCPs between the trophic guilds (section 4.2.3, Figure 29). As expected, piscivores had the highest levels of ΣOCPs, ΣHCH and ΣDDT. For HCB and mirex, the insectivores had higher levels and for Σchlordanes, concentrations were approximately equal in scavengers and piscivores (Figure 28).

As previously discussed, the accumulation of OCPs in fish, leading to high residue levels of POPs in fish tissue, is well-documented (Covaci *et al*., 2006). This increases the exposure of piscivore species. However, even within the piscivore guild there are also various trophic levels, depending on differences in prey. If a piscivore consumes larger sized fish that are often themselves scavenger or predators, higher POPs residues can be expected due to increased bio-magnification. The piscivore species, the African Darter, White-breasted Cormorant, and Heron *spp*., had significantly higher levels of *p,p*'-DDE, *p,p*'-DDT, ΣDDT and HCH isomers (one-way ANOVA, $p < 0.05$, section 4.2.1) compared to all other species although these levels did not necessarily differ significantly between each other ($p > 0.05$). However, the three piscivore groups included in the present study are on slightly different trophic levels. The White-breasted Cormorant is the largest species specially adapted to feed on benthic fish and capable of eating large prey. Some benthic fish are associated with sediment, often either filter feeding or scavenging. The African Darter has a similar diet and hunting strategy to the White-breasted Cormorant. The Darter is smaller though, and would be expected to feed on smaller fish. Heron *spp*. in turn, are normally more opportunistic, feeding on insects, frogs, molluscs, and fish. The diverse diet of heron *spp*, effectively means their exposure profile should be different from the other two predominantly fish-eating species. The White-breasted Cormorant had the highest levels of *p,p*'-DDE, β-HCH, γ-HCH and α-HCH, while the African Darter had the highest levels of *p,p*'-DDD. This shows a good agreement with the trophic level accumulation theory. The African Sacred Ibis had the highest levels of *p,p*'-DDT.

However, the pattern for mirex, HCB and Σchlordanes, did not support the trophic level hypothesis. HCB and mirex levels were highest in insectivores/omnivores followed by piscivores, while chlordane levels were approximately equal in scavenger and piscivores species. Levels for mirex were very low in all species. In freshwater ecosystems, there could be a high degree of homogeneity in background level concentration of mirex, compared to terrestrial ecosystems where there might be point sources such as disused consumer products where mirex was used as a flame retardant.

HCB is still released as an unintentional by-product of industrial processes as discussed in the section 5.2.1. This could lead to increased deposition on soil and plants, consequently leading to increased exposure in insectivores and omnivores. As expected, the granivores that are on the lowest trophic level also had correspondingly low levels of all OCPs, with the exception of mirex. When the congener profiles of the various OCPs were investigated on the basis of feeding guild (Figure 29), DDT was prominent in all guilds, and interestingly, the pattern

of pollutants were very similar in insectivores/omnivores and granivores. This could point to a similar exposure profile in these two guilds.

5.2.3. FEEDING HABITAT USE AND THE OCCURRENCE OF OCPs IN WILD BIRD EGGS

As with PBDEs, feeding habitat showed a statistically significant impact on the level of OCPs (one-way ANOVA, p = 0.00). The birds feeding in the terrestrial ecosystem had higher levels of all chlordane compounds and HCB, while aquatic feeding birds had higher levels of *p,p*'-DDE, ΣDDT, all HCH isomers, and ΣOCPs (Figure 31). The combined habitat user, the African Sacred Ibis, only had elevated levels of DDT compared to other habitat users (Figure 31).

Chlordane, HCH and HCB are still actively added to the environment in the area studied. Chlordane is used to control termites, and as termites are the main diet of many terrestrial insectivores, this may explain higher levels within these more terrestrially based species. In the case of HCB, it is deposited on soil and vegetation entering the terrestrial ecosystem, finally making its way into terrestrial biota.

HCH is used in agriculture as well as for control of ecto-parasites. However, low levels of γ-HCH indicate that HCH contamination originates from historical rather than current exposure. Thus HCH seems to be closely resembling the exposure profile of the metabolic by-product of DDT, namely *p,p*'-DDE, with higher concentrations in birds from the aquatic habitat. These species consist mainly of piscivores where biomagnification of these two compounds lead to increased exposure relative to the insectivores and granivores that represent the terrestrial habitat.

5.2.4. PCA OF OCPs IN SOUTH AFRICAN WILD BIRD EGGS

The relationships between various parameters and the concentration of OCPs were assessed using a PCA analysis (Figure 28). Factor 1 on the PCA-biplot explained 33% of the variance in the dataset and was a contrast between *p,p'*-DDE and β-HCH with negative scores and *trans*-nonachlor with positive scores. Both *p,p'*-DDE and β-HCH are indicators of historical applications of OCP pesticides (De Cruz *et al*., 1997; Konstantinou *et al*., 2000), while *trans*-nonachlor is a major constituent of technical chlordane (Dearth & Hites, 1991), indicating recent rather than historical use. The Crowned Lapwing was associated with *trans*-chlordane on the PCA-biplot with positive loadings. This species was separated from all other birds on the PCA-biplot, indicating a unique exposure pattern of OCPs confirming the results discussed in section 5.2.1. The distribution of bird species along factor 1 in the PCA-biplot also indicates the

association of the piscivore species (specifically the African Darter and White-breasted Cormorant) with *p,p'*-DDE and β-HCH with negative scores. These two species represent the top trophic level in the aquatic ecosystem, apart from the African Fish Eagle not studied here. Again, as species lower on the food web take up OCPs, they will metabolise parent molecules, retaining bio-accumulative metabolites. These metabolites will then be transferred to consumers on higher trophic levels. The Crowned Lapwing had positive scores and was strongly associated with chlordanes, indicating a unique OCP exposure or metabolic efficiency for this species (Figure 28).

Factor 2 explained 19% of the variance within the dataset and was mainly a contrast between α-HCH, γ-HCH, *cis*-nonachlor, and *cis*-chlordane with negative scores and *p,p'*-DDT and *p,p'*-DDD on the PCA-biplot (Figure 28). These compounds are more prevalent in technical applications and are associated with current applications of the pesticides. The separation of bird species along factor 2 was not very distinct. However, the Cape Sparrows were associated with *cis*-nonachlor and *cis*-chlordane, and the Southern Masked Weaver, African Darter, and White-breasted Cormorant with α-HCH and γ-HCH; all species having negative loadings. The African Sacred Ibis and Heron *spp*. with *p,p'*-DDT and *p,p'*-DDD had positive scores on the PCA-biplot. These distinct grouping may indicate species-specific exposure profiles and/or metabolic efficiencies of OCPs.

5.2.5. OCPs AND EGGSHELL THICKNESS

There were good correlations between the various OCP congener and isomers (Figure 32). The individual compounds that were correlated to one another indicate similar routes of exposure or mechanisms of accumulation (Stapleton & Baker, 2003).

As can be expected, the two major degradation products β-HCH and *p,p*'-DDE showed a strong correlation with one another, while the major degradation product of chlordane, namely oxychlordane, was correlated with, β-HCH and HCB. These correlations indicate that β-HCH shares a similar route of exposure or mechanism of accumulation with both chlordane and *p,p*'-DDE. Whereas, the lack of correlation of HCB and oxychlordane to *p,p*'-DDE, indicates that these chemicals have a different exposure and accumulation mechanisms as *p,p*'-DDE. This also corresponds to the current usage patterns for OCPs, where DDT is the only OCP that is not currently used or unintentionally produced in the study area. Low levels and patterns of HCH isomers seem to indicate historic exposure. However, since this product is still registered in South Africa it could be that usage has only recently declined, explaining a correlation with both the current and historic OCPs.

Additionally, correlations also indicate which congeners/isomers are related to the total concentrations of ΣOCPs. As can be seen in Figure 31, the ΣOCPs were correlated to the degradation products of DDT and HCH, but not to the active ingredients *p,p*'-DDT or γ-HCH, once again indicating historic rather than current exposure.

Although levels of ΣDDT were relatively high, there was no statistically significant linear correlation between eggshell thickness and OCPs, except for granivores. There was a statistically significant negative correlation between *p,p'*-DDD and eggshell thickness (Spearman rank order correlation; $p < 0.05$; $r = -0.88$) but not for *p,p'*-DDE, the compound commonly associated with eggshell thinning (Lundholm, 1997).

5.2.6. COMPARING OCPs IN WILD BIRD EGGS

OCP concentrations found in the wild birds studied were relatively high when compared to global levels reported in literature (Table 30). Maximum levels of ΣDDT were only exceeded by those measured in the New Territories of Hong Kong, in a heavily polluted river (Connell *et al*., 2003). The levels measured in piscovore species assessed in Hong-Kong were twice as large as the concentrations measured in the White-breasted Cormorant. Piscivore species in the current study had ΣDDT levels equivalent to the previous study from South Africa (Bouwman *et al*., 2008). Overall, the White-breasted Cormorant was the species with the highest levels of DDT when comparing the results from both studies, and it also represented the top trophic predator studied. The Crowned Lapwing had the highest levels of HCB as well as Σchlordanes.

The median values of HCB and Σchlordanes exceeded the levels measured in a single Crowned Lapwing by Bouwman *et al*. (2008) by 300 and 26 times, respectively. Samples collected in the study by Bouwman *et al*. (2008) originated from Parys while, in the current study, Cattle Egrets were collected in more industrially impacted sites, including an open area close a coal-fired power plant within the highly industrialised Vaal-Triangle. However, since only one egg was analysed, no firm conclusions can be drawn from this vast difference in OCP concentrations measured in these two South African studies. The chlordane levels reported for the Crowned Lapwing from this study is the second highest chlordane level compared to the literature consulted. The highest levels were reported for eggs of the Little Egret in the Hong Kong study (Connell *et al*., 2003). These levels were twice as high as that found in the eggs of the Crowned Lapwing from the current study.

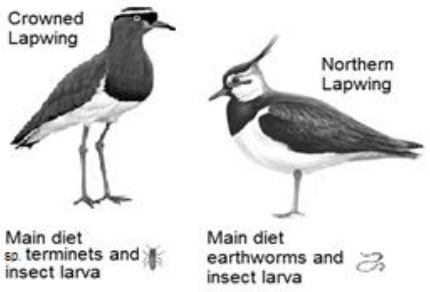


Figure 52: The Crowned Lapwing and Northern Lapwing from the *Vanellus* genus (adapted from Gibbon & Maclean, 2004; and RSPB, 2010.

Table 30: Mean/median concentrations of OCPs (ng g^{-1} lm) in bird eggs from South Africa, compared to levels of OCPs measured in eggs and fat tissue in other published studies

Common name	Scientific name	Feeding guild	Matrix	Years of sampling	n	HCB	∑HCH	∑DDT	∑Chlordanes	Study area	Source
Great Tit	*Parus major*	Insectivore	eggs	2000	38	9	11	601	6.8	Antwerp, Belgium	Van den Steen *et al*., 2006
Blue Herons	*Ardea herodias*	Piscivore	eggs	2000	10	120	ND	6470	550	British Columbia, Canada	Harris *et al*., 2003
Blue Herons#,^	*Ardea herodias*	Piscivore	eggs	2000	10	80	ND	2110	270	British Columbia, Canada	Harris *et al*., 2003
Little Egret	*Egretta gazella*	Piscivore	eggs	2000	20	ND	261	18 462	4308	Hong Kong	Connell *et al*., 2003
Black-crowned Night Heron	*Nycticorax nycticorax*	Piscivore	eggs	2000	20	ND	313	8955	462	Hong Kong	Connell *et al*., 2003
Herring Gull	*Larus argentatus*	Scavenger	eggs	2003	5	635	32	2 820	744	Hornøya, Northern Norway	Helgason *et al*., 2008
Black-legged Kittiwake	*Rissa tridactyla*	Piscivore	eggs	2003	5	625	34	806	336	Hornøya, Northern Norway	Helgason *et al*., 2008
Common Guillemots	*Uria aalge*	Piscivore	eggs	2003	5	467	14	962	46.8	Hornøya, Northern Norway	Helgason *et al*., 2008
Atlantic Puffin	*Fratercula arctica*	Piscivore	eggs	2003	5	665	29	1476	656	Hornøya, Northern Norway	Helgason *et al*., 2008
Northern Lapwing^	*Vanellus vanellus*	Insectivore	eggs	2006	14	50	7.3	97	205	Antwerp, Belgium	Dauwe *et al*., 2009
Great Tit	*Parus major*	Insectivore	eggs	2006	14	19	4.4	535	9.2	Antwerp, Belgium	Dauwe *et al*., 2009
Mediterranean Gull	*Larus melanocephalus*	Scavenger	eggs	2006	6	41	3.4	1116	89	Antwerp, Belgium	Dauwe *et al*., 2009
Black-necked Grebe	*Podiceps nigricollis*	Piscivore	fat	1992-1994	1	43	475	1391	40	Nakdong River, Korea	Choi *et al*., 2001
Great Knot	*Calidris tenuirostris*	Invertebrates	fat	1992-1995	1	101	82	159	48	Nakdong River, Korea	Choi *et al*., 2001
Sanderling	*Crocethia alba*	Invertebrates	fat	1992-1996	5	62	138	1007	126	Nakdong River, Korea	Choi *et al*., 2001
Greenshank	*Tringa nebularia*	Invertebrates	fat	1992-1997	3	15	80	1236	85	Nakdong River, Korea	Choi *et al*., 2001
Bar-tailed Godwit	*Limosa lapponica*	Piscivore	fat	1992-1998	5	121	36	657	38	Nakdong River, Korea	Choi *et al*., 2001
Black-headed Gull	*Larus ridibundus*	Scavenger	fat	1992-1999	7	117	229	2037	78	Nakdong River, Korea	Choi *et al*., 2001
Herring Gull	*Larus argentatus*	Scavenger	fat	1992-2000	4	149	231	2675	386	Nakdong River, Korea	Choi *et al*., 2001
Common Gull	*Larus canus*	Scavenger	fat	1992-2001	10	79	331	2564	108	Nakdong River, Korea	Choi *et al*., 2001
Common Tern	*Sterna hirundo*	Piscivore	fat	1992-2002	2	24	71	562	48	Nakdong River, Korea	Choi *et al*., 2001
Little Tern	*Sterna albifrons*	Piscivore	fat	1992-2003	3	173	90	6119	330	Nakdong River, Korea	Choi *et al*., 2001
Kentish Plover*	*Charadrius alexandrinus*	Insectivore	eggs	2008	8	ND	116	947	ND	Yellow River Delta, China	Gao *et al*., 2009
Common Coot*	*Fulica atra*	Omnivore	eggs	2008	4	ND	36	235	ND	Yellow River Delta, China	Gao *et al*., 2009
Common Tern*	*Sterna hirundo*	Piscivore	eggs	2008	9	ND	300	3715	ND	Yellow River Delta, China	Gao *et al*., 2009
African Darter	*Anhinga rufa*	Piscivore	eggs	2004-2005	14	70	1678	4407	149	South Africa	Bouwman *et al*., 2008
Reed Cormorant	*Phalacrocorax africanus*	Piscivore	eggs	2004-2005	3	40	79	6977	56	South Africa	Bouwman *et al*., 2008
Cattle Egret	*Bubulcus ibis*	Insectivore	eggs	2004-2005	20	12	13	375	13	South Africa	Bouwman *et al*., 2008
African Sacred Ibis	*Threskiornis aethiopicus*	Scavenger	eggs	2004-2005	2	16	40	1172	379	South Africa	Bouwman *et al*., 2008
Crowned Plover	*Vanellus cornonatus*	Insectivore	eggs	2004-2005	1	12	42	230	6	South Africa	Bouwman *et al*., 2008
Little Grebe	*Tachybaptus ruficollis*	Piscivore	eggs	2004-2005	1	18	16	958	6	South Africa	Bouwman *et al*., 2008
White-fronted Plover	*Charadrius marginatus*	Insectivore	eggs	2004-2005	1	9	15	390	5	South Africa	Bouwman *et al*., 2008
Kelp Gull	*Larus dominicanus*	Scavenger	eggs	2004-2005	1	52	13	880	8	South Africa	Bouwman *et al*., 2008
African Sacred Ibis	*Threskiornis aethiopicus*	Scavenger	eggs	2008-2009	16	6.9	12	320	26	South Africa	Present study
African Darter	*Anhinga rufa*	Piscivore	eggs	2008-2009	13	43	230	5050	75	South Africa	Present study
White-breasted Cormorant	*Phalacrocorax carbo*	Piscivore	eggs	2008-2009	4	22	240	8700	88	South Africa	Present study
Heron *spp.*	_	Piscivore	eggs	2008-2009	6	39	80	3700	53	South Africa	Present study
Cattle Egret	*Bubulcus ibis*	Insectivore	eggs	2008-2009	6	11	4.1	200	5.4	South Africa	Present study
Crowned Lapwing	*Vanellus coronatus*	Insectivore	eggs	2008-2009	6	309	33	84	1800	South Africa	Present study
Red-knobbed Coot	*Fulica cristata*	Omnivore	eggs	2008-2009	6	4.5	17	300	9.4	South Africa	Present study
Cape Sparrow	*Passer melanurus*	Granivore	eggs	2008-2009	9	12	6	32	4.7	South Africa	Present study
Southern Masked Weaver	*Ploceus velatus*	Granivore	eggs	2008-2009	8	14	36	394	41	South Africa	Present study

not oxychlordane; ^B-HCH; *All DDT and HCH associated compounds

In Belgium, concentrations of OCPs were measured in the Northern Lapwing (Figure 52), a species belonging to the same genus and with a similar diet and trophic position as the Crowned Lapwing. This species had similar OCP patterns than those found in the Crowned Lapwing, where chlordanes and HCB were the predominant OCPs in both species (Dauwe *et al*., 2009).

The highest ΣHCH concentrations were found in the piscivore species. Concentrations for the current study were in the same range as found elsewhere. In general, HCH levels were comparable with all literature reviewed with a relatively small difference when compared to DDT or chlordanes (Table 30). The most prominent levels are those measured in the African Darter previously reported (Bouwman *et al*., 2008), the highest of all reviewed literature, namely 1700 ng g^{-1} lm. This is five times higher than the values measured in the current study. The reason for this large variation is unknown. The samples in the previous study were collected from the Vaal River, Parys and the current study from Parys and Kempton Park. The individuals collected in Kempton Park would be expected to have higher levels due to historic production of lindane in close proximity to the collection area. This however, was not evident.

In general, OCP levels of the current study were higher than those measured in Norway, Belgium, and Canada and within the same range as those measured in China and Korea, but lower than the polluted site in Hong Kong (Table 30). Based on the comparisons alone, the levels of DDT and chlordane in wild bird eggs already indicate that there is cause for concern regarding South African birds, collected in the sampling areas of the current study. These levels of OCPs warrant further investigation to determine both abiotic environmental levels, as well as those in other animals with different trophic levels in both aquatic and terrestrial environments. Higher than expected levels of OCPs in wild birds located close to residential areas such as the Crowned Lapwing and the Southern Masked Weaver also indicate possible human exposure. Human exposure to OCPs should be monitored, both dietary and through contact with abiotic matrices.

5.2.7. POTENTIAL TOXICITY ASSESSMENT OF OCPs

OCPs are known to cause a myriad of negative physiological, toxicological, and behavioural impacts on birds (Allen & Thompson, 1996) including effects on eggshell parameters such as eggshell thinning (Nisbet, 1982). However, very few toxicity thresholds have been published for OCPs except for *p,p'*-DDE. Due to the low levels of mirex and HCB, these were excluded from the current toxicity assessment.

Threshold values for DDE as reviewed by Sample *et al*. (1996), Guruge *et al*. (1997), and Gao *et al*. (2009), vary widely with a range between 1 – 4 μg g^{-1} wm in eggs, depending on the species evaluated. Concentrations of 2.8 μg g^{-1} wm DDE have been associated with reproductive impairment of piscivore bird populations, while 1 μg g^{-1} wm has been linked to reduction of Heron survival (Connell *et al*., 2003). Eggshell thinning in Cormorants has been found to decrease hatching success at DDE concentrations of 4 μg g^{-1} wm. In the current study, the highest *p,p*'-DDE level (2.4 μg g^{-1}wm) was measured in an African Darter egg, collected in the Kempton Park region. This African Darter had a concentration twice that of the threshold for Heron *spp* of 1 μg g^{-1}wm. The second highest concentration was measured in a White-breasted Cormorant and an African Darter with a concentration of 0.9 μg g^{-1} wm. However, there was no statistically significant correlation of DDE with eggshell thinning in any of the species (section 5.2.4). Although the median concentrations for each species were below the threshold levels, these individual birds indicate that the reproductive integrity of wild birds in this study area, specifically predators, might be placed under additional stress through the high levels of DDE.

There are limited data on the effect of HCH on hatching success or embryo mortality in wild birds. However, β-HCH has been linked to endocrine disruption (Willett *et al*., 1998). Organisms are exposed to multiple types of EDCs at any given time (Ottinger *et al*., 2005), causing multiple effects that are not necessarily singularly large enough to cause a gross physiological effect. When combined, these effects could, on a chronic basis, cause extra stress on an organism, leading to negative health effects. Levels measured during this study were below the threshold level of 5 – 10 μg g^{-1} wm for ΣHCH, which has been used previously as a NOEL (Goa *et al*., 2009). The species with the highest concentration of ΣHCH was the White-breasted Cormorant (16.82 ng g^{-1} wm) with a level 300 times lower than the NOEL. This indicates that for birds in the current study, exposure to HCH was below levels that would be expected to cause detrimental health effects. However, since this compound acts as an endocrine disrupter, as does *p,p*'-DDE and other environmental pollutants, the combined effects remain unknown.

Chlordane has been linked to mass death of birds in the USA (Stansley *et al*., 2001). Concentrations of Σchlordanes in the main insect prey of the various affected birds species ranged between 0.02 – 15 μg g^{-1} wm. In seemingly healthy birds, concentrations of oxychlordane ranged between 0.1 – 0.5 μg g^{-1} wm, while dead bats contained oxychlordane concentrations between 0.4 – 5 μg g^{-1} wm. Although bats cannot be compared to birds, it does give an indication of chlordane toxicity in vertebrates. However, no studies on sub-lethal effects or threshold values could be found. From available data, a NOEL of 0.5 μg g^{-1} wm was

assumed. The levels found in the Crowned Lapwing was 18 times higher than any other species, and this concentration was five times lower that the proposed NOEL. This indicates that chlordane is currently not a threat to birds in the areas studied.

SECTION 3
PCBs IN WILD BIRD EGGS IN INDUSTRIALISED CENTRES OF SOUTH AFRICA

Although the industrial uses of PCBs have been phased out since the 1970s, this compound remains ubiquitously present in almost all environmental compartments. This is not only due to remaining historic uses, but also due to the formation of PCBs as unwanted by-products of industrial and thermal processes. PCBs were detected in all egg samples with 30 PCB congeners of the 34 analysed being present in more than 80% of samples.

5.3.1. CONGENER PROFILES OF PCBs IN WILD BIRD EGGS

The most prevalent congeners present were CB-138, -153, and -180. This pattern has been seen in a many biological matrices including birds' worldwide (Boumphrey *et al*., 1996; Mora, 1996; Humphrey *et al*., 2000; Dip *et al*., 2003). The high levels of these congeners in organisms are usually attributed to their recalcitrant nature in the environment, bioaccumulative nature, and low metabolic degradation potential (McFarland & Clarke, 1989; Borgå *et al*., 2005; Antoniadou *et al*., 2007; Luo *et al*., 2009a). Current releases through products that are still in service, together with unintentional production, are still factors in PCB presence in the environment and biota (Breivik *et al*., 2002). In general, it is assumed that levels that are skewed to higher PCB congeners are linked to historic applications, whereas the presence of lighter congeners are attributed to current sources (Breivik *et al*., 2002). The presence of measurable levels of PCBs of almost all congeners indicates current sources in the sampling areas.

The PCBs are often found at higher concentrations in piscivore species due to their position in the food chain, and the increased aquatic exposure of their prey to organohalogen compounds (Zimmerman *et al*., 1997). Therefore, as expected the highest concentrations of PCBs were measured in the eggs of piscivore species, the White-breasted Cormorant and African Darter (Table 22).

The congener profiles of PCBs were further elucidated through PCA analysis (Figure 35). Factor 1 explained 32% of variance in the dataset and was a contrast between lower chlorinated species with positive scores and higher chlorinated species with negative scores. Piscivores were associated with higher chlorinated PCBs, while insectivores/omnivores and granivores were associated with lower chlorinated PCBs on the PCA-biplot. Birds on a higher trophic level would be expected to have higher chlorinated PCBs (Ólafsdóttir *et al*., 2001). Prey species have

higher levels of lower chlorinated congeners. However, since lower chlorinated PCBs are more readily metabolised, higher PCBs are more likely to be transferred 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 that consume herbivores will take up these congeners.

Factor 2 explained 22% of the variance within the dataset and was a contrast between the more metabolically active group III PCBs with positive scores and the less metabolically active group I PCBs with negative scores (Figure 35). Group III PCBs have vicinal hydrogen atoms in the *ortho-meta* positions and are more readily degraded through CYP 1A enzyme linked reactions. Group I PCBs are persistent due to the lack of vicinal hydrogen atoms (Borgå *et al*., 2005). Species were not strongly separated along factor 2 in the PCA-biplot. However, the Southern Masked Weaver and Red-knobbed Coot with positive loadings were separated from the heron *spp*. with negative loadings. This indicates that the Heron *spp.* eggs contained more persistent PCB congeners when compared to Southern Masked Weaver and Red-knobbed Coot.

5.3.2. DISTRIBUTION OF LOWER AND HIGHER CHLORINATED PCBs IN WILD BIRD EGGS

When investigating PCB distribution according to the number of chlorine atoms present on the biphenyl ring, it became clear that there was a statistically significant difference between species, feeding guilds, and habitats (one-way ANOVA; $p < 0.05$; section 4.3.1: Table 23; Figures 36 and 37). As with previous studies concerning wild bird eggs, hexa-CB was the predominant group in all species (Table 23), likely due to the high persistence associated with these PCBs (Luo *et al*., 2009a). The order of PCB abundance that followed hexa-CB differed between species. Although PCBs are resistant to metabolism, congeners are metabolised by various degrees, with resistance to degradation increasing with the degree of chlorination. However, the extent to which a PCB will be metabolised seems to be species-specific (Henry & De Vito, 2003).

There was an increase in PCB levels in eggs corresponding to the trophic levels of the parent bird. Piscivores had the highest levels, followed by scavengers and insectivores/omnivores (Figure 36). 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). For feeding habitat, the higher chlorinated congeners seemed predominant in aquatic and combined habitat bird species, while

lower chlorinated congeners, based on levels in eggs, were bio-available in both aquatic and terrestrial feeding habitats (Figure 37). The difference between the higher and lower chlorinated PCBs could, however, also imply differential exposure to various metabolic groups of PCBs. Additional research is required to elucidate this dictomy.

5.3.3. METABOLIC PCB GROUPS IN WILD BIRD EGGS FROM SOUTH AFRICA

Group I PCBs are categorised as the most persistent PCBs due to resistance to enzymatic metabolism. The lack of vicinal hydrogen atoms increases steric interference when inserting the oxygen atom as the first step in biotransformation (McFarland & Clarke, 1989; Boon *et al*., 1997). Group I PCBs was predominant in all eggs with the exception of the Red-knobbed Coot where group III PCBs were dominant (Figure 35). From Group II to V, PCBs become more biodegradable due to the steric configuration of the molecule, the presence of vicinal H atoms, and often a decreasing number of chlorine atoms (Borgå *et al*., 2005). In most species the concentration of group V and VI in eggs was negligible in comparison to group I to III (Figure 38) Levels of group V and VI PCBs also did not differ statistically significantly between species (one-way ANOVA, $p > 0.05$, section 4.3.2). The statistically 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).

When looking at the different enzymes induced per PCB group between the species, PB-type inducers were predominant in all species (Figure 39). PB inducible enzymes catalyze the biotransformation of PCBs through facilitating oxygen insertion into the molecule (McFarland & Clarke, 1989). These PCBs are classified as less toxic than the mixed type inducers or DL-PCBs. In general, there were higher levels of mixed-type inducers as well as DL-PCBs in piscivores and scavengers, indicating increased risk to these bird species (Figure 36). Due to the high levels found in piscivores, one-way ANOVA results between feeding guilds and feeding habitat types could not be used (section 4.3.2). These aspects need further research for South African conditions.

5.3.4. PCB-CONGENERS AND EGGSHELL THICKNESS IN WILD BIRD EGGS

There was a strong correlation between the different PCB congeners (Figure 40). CB-138 and -114 were statistically significantly correlated to 94% of all other PCB congeners, while CB-180 was correlated to 90% of congeners (possibly due to high concentrations). Combined with the high prevalence of CB-138 and -180, these congeners are ideal monitoring PCBs in the

South African environment. However, due to the toxicological importance of PCB-153 (Strathmann *et al*., 2006), it should also be included in residue monitoring programmes.

Although the previous study by Bouwman *et al*. (2008) indicated eggshell thinning in the African Darter corresponding to the high levels of PCBs, these findings were not confirmed in this study. The only species that showed a statistically significant decrease in eggshell thickness corresponding to increased levels of PCBs in eggs were the Crowned Lapwing and granivore *spp*. Due to the relatively small sample size the trend can be identifies, but no conclusions can be drawn therefrom.

5.3.5. COMPARISONS OF PCBs FOUND IN WILD BIRD EGGS

In general, PCB concentrations in wild bird eggs studied were lower than that in reviewed literature (Table 31). Levels were comparable between the current study and those reported by Bouwman *et al*. (2008). PCB levels in 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 piscivores from Korea (Choi *et al*., 2001). All other piscivores in the literature review were in the same range 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 – 53% less 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.

5.3.6. POTENTIAL TOXICITY ASSESSMENT OF PCBs IN WILD BIRD EGGS

Although PCBs were of the first chemicals directly linked to decreases in wildlife populations, there is still a lack of data on the relative importance of non-dioxin like PCBs in toxicity assessments of wild bird populations (Henry & De Vito, 2003).

Reproductive failures have been linked to ΣPCBs concentrations of 1.6 – 10 $\mu g.g^{-1}$ wm in cormorant and heron species (Antoniadou *et al*., 2007). Decreased hatchling success has been reported at concentrations of 1 – 25 $\mu g.g^{-1}$ wm for chickens, cormorants and eagles (Brunström & Halldin, 2000). Mortality in birds has been associated with concentrations between 90 – 470 $\mu g\ g^{-1}$ wm (Guruge *et al*., 1997). Considering the above-mentioned data, the NOEL of 4 $\mu g\ g^{-1}$ wm in eggs as proposed by Brunström & Halldin (2000) was used to assess the risk of birds studied to PCB.

Table 31: 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	Nr 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	Connell *et al*., 2003
Black-crowned Night Heron	*Nycticorax nycticorax*	Piscivore	eggs	2000	20	3433	NA	Hong Kong	Connell *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

The highest levels of ΣPCBs were measured in a White-Breasted Cormorant egg (290 ng g^{-1}, Table S7). This level was 13 times lower than the NOEL of PCBs, indicating low risk of reproductive failure or mortality from the current level of PCBs measured. However, there are sub-lethal effects that cannot be as easily assessed. Birds with increased levels of PCBs have been linked with behavioural impairment (Bustnes *et al*., 2001) that can cause variation in normal hatching and rearing behaviour. Sub-lethal effects of PCBs also include altered hormone function and increased stress responses (Fry, 1995; Langer, 1998; Dawson, 2000). Although levels measured in South African bird eggs were low, these sub-leathal effects may still be affecting the health of bird populations and need to be assessed under African conditions.

SECTION 4
ORGANOHALOGENS IN WILD BIRD EGGS FROM INDUSTRIALISED CENTRES OF SOUTH AFRICA

The last number of years has seen a clear shift in the contamination pattern of organohalogens globally. In the 1960s and 1970s, there were high concentrations of DDT and low concentrations of PCBs (Custer *et al*., 1983). This pattern has now shifted to PCBs being the predominant chlorinated chemical in many parts of the world (Pastor *et al*., 1995; Guruge *et al*., 1997; Braune, 2007). However, this pattern can be affected by the major activities in the local environment, as well as historic applications and usage profiles. If an area's predominant activity was agricultural, then the predominance of OCPs above the more industrially associated PCBs is expected (Sakellarides *et al*., 2006), and *vice versa*.

The current main study areas where wild bird eggs were collected were highly impacted by industry. However, the nesting sites were in areas with less human activity such as clearings, recreational areas, along riverbanks and in wetlands. The exceptions were Crowned Lapwings and Cape Sparrows eggs collected in residential areas or directly alongside large industries. During this study, OCPs contributed the greatest to the total organohalogen burden in all species except the African Sacred Ibis and granivores (Figure 41). The African Sacred Ibis had slightly higher levels of PCBs than OCPs. However, this could be due to the adapted diet of the species. On the other hand, in granivores, BFRs contributed the greatest to total organohalogen compounds. Due to the highly industrialised nature of the area, this profile was relatively surprising. Additionally, the compound contributing the largest portion to OCPs was *p,p*'-DDE that has been deregistered since the 1970s. The legacy of DDT usage in South Africa is very prominent in these sites, but current loadings of DDT cannot be excluded. Although DDT can no longer legally be obtained, there is the possibility of illegal as well as atmospheric transport from areas where DDT is actively applied for malaria control, as discussed in section 5.2. There is also another possibility - the use of dicofol, which had been registered for use in South Africa for quite a long time (Nel *et al*., 2002).

As mentioned previously, dicofol is an acaracide used on numerous crops including fruit and cotton (Clark *et al*., 1990; Qiu *et al*., 2005) as well as in residential gardens (Nel *et al*., 2002). DDT as well as DDE are contaminants often found in technical dicofol (Yang *et al*., 2008). Additionally impurities within dicofol can also be degraded to DDE (Yang *et al*., 2008) as these compounds follow similar degradation pathways as DDT. The current or

historic use of dicofol in the study area could thus contribute to high levels of DDE found in wild birds.

The White-breasted Cormorant and African Darter had the highest levels of OCPs and PCBs, while the African Sacred Ibis had the highest levels of BFRs (Figure 41). There were large differences in the concentrations of ΣPCBs between species. This variation is likely due to differences in habitat, feeding behaviour, diet, and proximity to point sources.

Cluster analysis was used to investigate the relationship between the main organohalogen groups between wild bird species (Figure 42). The cluster arrangement as shown in Figure 42 was the same for all species with the exception of the Cape Sparrow. The majority of species showed two clusters: OCPs and PCBs clustered together, with BFRs separate (Figure 42). It can be interpreted that for these species OCPs and PCBs have similar routes of uptake, different from BFRs. This pattern is reversed for the Cape Sparrow. In this species, BFRs grouped with PCBs separate from OCPs (Figure 42). For most organohalogen compounds, the main uptake route is through dietary intake. In fish, uptake can occur over the gill surface. However, for PBDEs, dust and air particulate matter seems to be an important route of uptake for sparrows. Commercial products leach BFRs into the environment, contaminating indoor dust and air (Harrad *et al*., 2008). The Cape Sparrow has adapted to live in close association with humans, for instance large shopping centres. Sparrows will even scavenge food scraps in homes. These associations may increase exposure to indoor dust and air, leading to increased exposure to BFRs. The sparrow's low position in the food web on the other hand, translated into lower levels of OC compounds compared to other species. These factors lead to a pollutant profile in eggs consistent with species behaviour and guild (Guruge *et al*., 1997).

5.4.1. PCA OF ORGANOHALOGEN COMPOUNDS IN WILD BIRD EGGS

In the PCA (Figure 43), factor 1 explains 34% of the variance in the dataset and is a contrast between BDEs with negative scores and PCBs with positive scores. In the PCA-biplot, the African Sacred Ibis, granivores, insectivores and omnivores associated with the BDEs and light PCBs with negative loadings and the piscivores associated with the heavier PCBs. Lighter PCB congeners are prone to long-range transport and volatilisation (Ockenden *et al*., 2003). Additionally, these PCB congeners have higher levels in water and prey than in predatory birds, since they are more readily metabolised (Antoniadou *et al*., 2007). This explains the compound associations on the PCA-biplot with the low trophic levels species, the granivores, insectivores, and Red-knobbed Coot. The heavier PCBs were associated with the heron *spp*., African Darter, and White-breasted Cormorant. All three of these species are piscivores, feeding higher on the food web and more prone to accumulate the heavier, more metabolically stable PCB congeners. The mono-*ortho* PCBs were the

exception, these two low molecular weight PCBs were associated with the African Darter on the PCA-biplot.

The African Sacred Ibis and Black-headed Heron were associated with the heavier PBDE and HBCD (Figures 43 and 44), indicating exposure to current-use BFRs. However, BDE-183 was also present in the grouping, indicating exposure to technical octa-BDE (Guardia *et al*., 2006). The granivores, insectivores, and Red-knobbed Coot were associated with the lighter PBDEs, BDE-28, -47, and -99. Lighter PBDEs can be a byproduct of the penta-technical mixture or a metabolic product of the heavier PBDEs (La Guardia *et al*., 2006). Additionally, BDE-47 and -99 were associated with air and dust transport of PBDEs (Van den Steen *et al*., 2007; Law *et al*., 2008).

Factor 2 explained 17% of the variance in the data (Figures 43 and 44) and consisted of a contrast between a mixture of OCPs, PCBs, and BDE-47 with negative scores, and a mixture of PCBs, BDEs and *p,p'*-DDT with positive scores. The negative scores consisted of chordanes, α-HCH, and HCB, together with the lower chlorinated PCBs and lower brominated BFR congeners. The positive scores consisted of the higher chlorinated/brominated PCBs and PBDEs, HBCD, and DDT. The scavengers and piscivores grouped along side the higher congeners of PCB and BFRs as well as the DDT, while granivores, insectivore/omnivores, African Darters, and White-breasted Cormorants grouped alongside the lower chlorinated/brominated PCB and PBDE congeners, as well as the other OCPs on the PCA-biplot (Figures 40 and 41). The two piscivores associated specifically with β-HCH, a metabolite of lindane. The grouping of the organohalogens in the PCA-biplot seems to be dictated by the individual chemicals physiochemical properties.

Factor 3 explained 11% of the variance within the dataset (Figure 44) and was a contrast between OCPs (*trans*-nonachlor, oxychlordane, mirex, HCB and *cis*-nonachlor) and higher chlorinated PCBs with positive scores and PCBs (CB -31, -74, and -28) PBDEs and HBCD with negative scores. When factor 3 was plotted against factor 2, the Crowned Lapwing separated from all other species on the PCA-biplot. This indicated a unique exposure profile that was explained by the high prevalence of chlordanes and HCB in this species as discussed in section 5.2.3. It has also been found that the lower chlorinared PBDEs have similar physiochemical properties as PCBs and this suggests comparable bioaccumulation potential in the environment (Stapleton & Baker, 2003).

There was no distinct separation according to geographical distribution for compounds or bird species on the PCA-biplot. This however, could be due to the relative proximity of the sampling sites. Birds are never completely localised to a particular area; they are able to fly relatively long distances in search of suitable food sources. Moreover, when looking at levels in piscivorous birds it must be taken into account that fish themselves can move long distances and take up pollutants at areas far from the place of their predation. Birds are

capable of traversing large distances while foraging for food. In the case of food shortages, many species including the Cattle Egret, African Darter, and African Sacred Ibis will become locally nomadic, travelling to find more suitable habitat. The sampling sites were all within 150 km of one another, connected by the Vaal River Catchment in many of the study sites. These factors lead to geographical crossover, decreasing site specific effects on the congener profiles of organohalogenated compounds.

5.4.2. RELATIONSHIPS BETWEEN TROPHIC LEVEL AND FEEDING HABITAT AND ORGANOHALOGENATED COMPOUNDS IN WILD BIRD EGGS

Food webs in their most basic form consist of four defined levels - producers, herbivores, predators, and detrital feeders (Braune *et al*., 1999). However, food webs are not simplistic since there are multiple interactions such as opportunistic feeders, different levels of predation and omnivores that will cause mixed exposure profiles to pollutants. As expected, there was a statistically significant (one-way ANOVA, $p = 0.00$) difference in the pattern of OCs within the various feeding guilds (Figure 45).

In the scavenging guild, the pattern of contaminants in wild bird eggs were PCBs>OCPs>BFRs. Scavenging species are opportunistic feeders that play an important role in the flow of energy and matter within the ecosystem, and as such scavengers can be susceptible to pollutants (Lemus *et al*., 2009). The highest levels of BFRs were measured in the scavengers. However, these levels are more likely attributed to the area of scavenging that includes refuge tips than the trophic level.

The highest levels of OCPs and PCBs were found in piscivore eggs as discussed in section 5.2.2. Insectivores/omnivores had the same pattern of organohalogen compounds than piscivores, whereas granivores differed from all other species. In the granivores, BFRs were the predominant organohalogen compound present. As discussed in section 5.1.2, granivores have increased contact with humans compared to the other species sampled. In South Africa where BFRs are not actively produced, the assumption can be made that the main source of these chemicals to the environment will be through release from products and waste. Through this process, house dust and air will be contaminated with BFRs and due to their close association with humans, granivores will have higher exposure compared to other wild bird species, resulting in the elevated levels found in their eggs. The quantity of BFRs imported into South Africa is not currently known.

The concentration of organohalogen compounds differed statistically significantly between the different habitat preferences of the studied species (one-way ANOVA, $p < 0.0001$, Figure 46). Aquatic birds had higher levels of OCPs and PCBs. The levels of POPs measured in biological matrices are not only linked to the amount released into the environment, but also to intrinsic properties of the pollutants. Depending on the chemical

properties, pollutants can be classified as flyers (pollutants that will travel in the air); swimmers, (POPs that are more water soluble and can be circulated through rivers, streams and ocean currents); and pollutants that are more inclined to deposit and adhere to soil and sediment (Lohmann *et al*., 2007). These characteristics will also determine the partitioning of pollutants between the different environmental compartments. To study this effect in more detail however, more eggs from species per habitat preference will need to be sampled.

During this study the various feeding habitats were not represented by birds in corresponding niches. Therefore, no firm conclusions on the partitioning of POPs between the different habitats could be drawn.

However, the differences in organohalogen levels in eggs between birds in various guilds and habitat does indicate the importance of trophic level as well as exposure profiles to the bioaccumulation of organohalogens in wild birds.

5.4.3. RATIOS BETWEEN ORGANOHALOGEN GROUPS IN WILD BIRD EGGS

The ratios between organohalogens can provide information about difference in sources or exposure profiles between areas and species. The DDE/ΣPCB ratio differed between species and sites (Table 24). In general, granivores, the Crowned Lapwing and the African Sacred Ibis had DDE/ΣPCB ratios < 1, indicating a stronger exposure to PCBs than OCPs where as all other species had DDE/ΣPCB ratios > 1. This indicates bird associations with anthropogenic activity dominated by PCBs rather than OCPs. This could be due to diet, or in the case of the Crowned Lapwing, species-specific variation in metabolic activity. Leaching of PCBs from refuge tips and from households could cause differential exposure. Additionally, these species are found close to industries, including power generation and steel making plants, as well as petro-chemical industries; all are known sources of PCBs (Nadal *et al*., 2007; Choi *et al*., 2008; Sahu *et al*., 2009).

Ratios > 1 indicate greater exposure to OCPs and specifically DDT and its metabolites. The highest ratio was found in aquatic bird's eggs, specifically the piscivores (Table 24), contrary to the findings in Bouwman *et al*. (2008), where terrestrial species had higher ratios. The ratios were also higher in Kempton Park than any other sampling site (Table 24). Kempton Park was expected to have higher loadings of DDE due to the past production of technical DDT in the area (Osibanjo *et al*., 2002).

The median ratio of ΣPBDE/ΣPCB was < 1 for all species except the granivores, which have a different exposure profile as discussed in section 5.1.2 and 5.4. The higher levels of PCBs compared to PBDEs in the eggs are likely due to their higher environmental levels. PBDEs are readily degradable through photolytic degradation pathways compared to PCBs. However, it has been postulated that birds differ in their metabolic capacities in regard to

these two chemical groups (Stapleton & Baker, 2003). This explanation is still speculative with no conclusive evidence. The sampling areas with the highest ΣPBDE/ΣPCB ratios were Soweto and Vanderbijlpark, indicating increased sources of PBDEs (Table 24). In Vanderbijlpark, this could be attributed to the steel manufacturing industry where insulating material is used (section 3.2). The site in Soweto had a wide diversity of possible contamination sources, including industries, residential activities, refuge, and sewage, all factors that could increase levels of organohalogen compounds in the area.

5.4.4. THE RATCLIFFE INDEX

The Ratcliffe index of shell thickness was used to determine eggshell thickness (Burnham *et al*., 1984; Davies & Randall, 1989; Dirksen *et al*., 1995) before accurate micro-scale callipers became commonplace. This index still has advantages since it can be used to compare different species and studies when the necessary data of circumference and length is known. This is not possible without direct measurements since the eggs of species vary significantly from one another.

The resulting index of the current study was compared to available literature for South Africa (Mundy *et al*., 1982; Snelling *et al*., 1984; Davies & Randall, 1989). The results indicated that current levels were not different from those obtained more than 20 years ago (Figure 47) but a statistically significant difference in the eggshell index was found when the Cape Vulture was excluded.

When considering the small difference in the Ratcliff index values, between the current and historic studies, with the fact that DDT is no longer actively used, it may be supposed that chemicals other than DDT may now be dominant in reducing eggshell thickness. Other chemicals such as PBDEs have since been added to the environment, and these chemicals have endocrine disrupting properties (Darnerud *et al*., 2001; Vonderhede *et al*., 2008). The combined effect of the current-use and legacy man-made chemicals seem to have the same combined effect as when DDT was still used in agriculture. The experimental design was not originally meant for this comparison and the small samples sets are too small to derive any definite conclusions. However, more work on historical samples should be done.

SECTION 5
ORGANOHALOGENS IN CHICKEN EGGS FROM SOUTH AFRICA

5.5.1. ORGANOHALOGEN COMPOUNDS IN CHICKEN EGGS

The chicken eggs collected in the low-income residential areas within the study area had low levels of BFRs (Table 26) with BDE-209 having the highest detection frequency of 60% of the eggs. Chicken eggs generally only had measurable levels of BDE-153, -183, -207, -208, -209, and HBCD. High levels of BDE-183, -209, and HBCD indicate the use of technical octa-BDE mixtures as well as the use of Deca-BDE and HBCD (La Guardia *et al*., 2006), while high levels of nona-BDE further point to the presence of deca-BDE in the environment of the chickens, since BDE-209 has been found to primarily degrade to nona-BDE congeners (Luo *et al*., 2009b).

Backyard chickens live in close association with the environment and are thus considered good indicators of the contamination levels in the areas they inhabit (Van Overmeier *et al*., 2006). Therefore, it can be assumed that humans living in these areas are also exposed to higher levels of technical octa- and deca-BDE mixtures of BFRs as well as HBCD, rather than to the lower brominated technical mixture.

In general, eggs collected in the Vanderbijlpark area had higher levels of BFRs than commercial eggs as well as the backyard eggs collected in Coalbrook, Sasolburg (Figure 48). Levels in backyard eggs collected in Vanderbijlpark was statistically significantly higher for BDE-209, -183, -207 and ΣPBDEs compared to commercial eggs (Kruskal Wallis ANOVA, $p < 0.05$), while backyard eggs collected in Coalbrook did not differ significantly from commercial eggs (Kruskal-Wallis, $p > 0.05$, section 4.5.1). Eggs collected in Vanderbijlpark also had significantly higher levels of BDE-207 compared to Coalbrook (section 4.5.1). Industries in Vanderbijlpark are mainly associated with steel manufacturing. This includes the production of pipes insulated with fusion-bonded polyethylene and epoxy linings, as well as insulated wire and insulating materials (PVC and XPLE), all possible sources of BFRs.

PBDE levels found in this study ranged between NDs – 160 ng g^{-1} lm. These levels were 5 times higher than those measured in Belgium (ND – 32 ng g^{-1} lm) in home-produced eggs (Covaci *et al*., 2009) and 27 times lower than levels found in the liver of domestic fowl from an e-waste recycling area in China (Luo *et al*., 2009b). An interesting observation was that although the concentration of PBDEs did not affect the eggshell thickness, there was a negative correlation to the length and circumference of the egg. This needs further research.

DLCs were found in all chicken eggs sampled, with concentrations ranging between 76 – 5 500 pg g^{-1} wm. The predominant congeners for the various groups of DLCs were

OCDD, HpCDD, PeCDF, TCDF, and CB-118. The highest contribution to the concentration of DLCs was from mono-*ortho* CBs (Figure 50). The pattern of dioxin contributions were the opposite of those for BFRs, with the highest concentration measured in commercial eggs from Pretoria. However, when mono-ortho PCBs were not considered, the highest concentrations occurred in Vanderbijlpark (Figure 49).

When concentrations were converted to TEQ values, levels ranged between 0.7 – 6.4 pgTEQ g^{-1} lm (Table 27). The concentrations per egg were as follows; Vanderbijlpark > Pretoria commercial egg > Bloemfontein commercial egg > Coalbrook. Vanderbijlpark houses one of the largest steel industries in Africa. Steel production is a known source of DLCs through the iron ore sintering process as well as high temperature used in metal processing (Buekens *et al*., 2001, Anderson & Fisher, 2002). The levels of DLCs in commercial eggs were mainly due to CB-118 that contributed more than 60% of the total TEQ in commercial eggs from Pretoria and Bloemfontein. In a study conducted by Nieuwoudt *et al*. (2009) on the occurrence of DLCs in the Vaal-Triangle South Africa, CB-118 was also found to be prevalent. Possible sources of CB-118 include commercial PCB preparations as well as incineration processes (Nieuwoudt *et al*., 2009). CB-118 levels found in commercial eggs could either come from PCBs leaching from old equipment that is still in use, or because of waste incineration processes. In the backyard eggs from Vanderbijlpark, the predominant congeners was CB-126 contributing more than 30%, and 1,2,3,7,8-PeCDD that contributed approximately 25% to the total TEQ. CB-126 is considered the prevalent congener associated with combustion sources (Kim *et al*., 2004), while 1,2,3,7,8-PeCDD is often a predominant congener (Bhavsar *et al*., 2007; Moon *et al*., 2008), also assiociated with combustion sources (Masunaga *et al*., 2003). Levels in this study (0.7 – 6.4 pgTEQ g^{-1} lm) were 16 times lower than concentrations measured in home-produced eggs from Belgium (3.29 – 95.35 pgTEQ g^{-1} lm; Van Overmeire *et al*., 2009) and 3 times higher than commercial eggs from Sweden (2.14 pgTEQ g^{-1} lm; Darnerud *et al*., 2006). As with PBDEs, there was a negative correlation between the concentration of DLCs and the circumference of the egg. However, for dioxins there was also a negative correlation to the eggshell thickness (Spearman rank order correlation; $p < 0.05$).

OCPs were detected in all chicken eggs analysed, with concentrations ranging between 0.2 – 20 ng g^{-1} (Table 28). HCB was the predominant OCP in eggs from Vanderbijlpark as well as Coalbrook, while HCH was the dominant OCP in commercial eggs (Figure 51). Levels in eggs collected in Vanderbijlpark were statistically significantly higher for, HCB, oxychlordane, *trans*-nonachlor, *cis*-nonachlor, *p,p'*-DDE, and ΣDDT, when compared to both commercial eggs as well as eggs from Coalbrook (section 4.4.2.2). The mainly metabolic degradation products β-HCH and *p,p*'-DDE, were the predominant

compounds for ΣHCH and ΣDDT, respectively. The presence of measurable levels of both γ-HCH and *p,p'*-DDT, does, however, indicate a more recent exposure to DDT and HCH. *Trans*- and *cis*-chlordane were the predominant chlordane's, indicating recent exposure, when the low levels of their metabolites oxychlordane are taken into account (Bondy *et al*., 2003). Mirex was not detected in any of the samples, except a single commercial egg from Bloemfontein (Table 28).

The congener patterns of OCPs indicate current exposure to DDT, lindane, and chlordane. For chlordane, HCH, and HCB, current exposure can be expected due to active use of the first two compounds and non-intentional production of HCB (as discussed in Section 5.2.1). However, the presence of DDT is unexpected since active application is not permitted in the area. As discussed by Bouwman *et al*. (2008), this could be due to illegal use, leaching of old stockpiles, as well as atmospheric transport from the Northern areas of South Africa. As can be seen in Table 32, South African levels were comparatively low for DDT and HCH, but higher for chlordane's and HCB.

Table 32: Comparative values of OCPs in chicken eggs (ng g^{-1} lm)

Area	HCB	ΣHCH	ΣChlordanes	ΣDDT	Reference
Coalbrook, Sasolburg	3.23	1.50	1.00	2.56	Current study
Vanderbijlpark	45.55	3.76	23.54	34.93	Current study
Commercial	1.78	4.08	0.91	1.05	Current study
Jordan	ND	110.00		720	Ahmad *et al*., 2010
Belgium	ND	ND	ND	2165	Windal *et al*., 2009
Sweden	1.37	0.56	0.65	6.59	Darnerud *et al*., 2006

5.5.2. EXPOSURE TO POPS THROUGH THE CONSUMPTION OF BACKYARD CHICKEN EGGS

Protein is an essential nutrient and inadequate dietary protein results in poor growth, development, low resistance to infection, and generally coincides with a shortage of other essential nutrients (Van Oostdam *et al*., 2009). This makes food security in low-income areas essential, especially when additional factors such as the prevalence of AIDS is brought into consideration. In South Africa, backyard poultry is an important food source and essential in providing protein to low-income families. However, dietary exposure is an important route of human exposure to organohalogen compounds (Van Oostdam *et al*., 1999; Moon *et al*., 2009). In non-occupationally exposed individuals, food can be the predominant source of organohalogenated compounds (Wilhelm *et al*., 2002). Additionally, there are generally higher concentrations of organohalogen compounds in home- or backyard-produced eggs compared to commercially produced battery eggs due to increased contact with soils (Windal *et al*., 2009).

Chickens are also known to scavenge snails, insects, earthworms, grass seeds, as well as kitchen leftovers (Swatson *et al*., 2009). Chickens are also geophagous, consuming soil and small stones to assist in digestion. The diet and behaviour of chickens lead to increased exposure to POPs. The ingestion of chicken eggs can thus contribute to a person's total intake of organohalogen compounds.

Exposure to POPs due to dietary intake can be determined by comparing tolerable daily intakes (TDIs) to the average daily intake (ADI) (Wilhelm *et al*., 2002), thereby determining the percentage a specific food source will contribute to the total dietary exposure to organohalogens. All risk assessment calculations were based on the U.S. Environmental Protection Agency (EPA), Public Health Assessment Guidance Manual: Calculating exposure doses (2005). The ADIs were calculated by determining the exposure dose (Table 33) from ingestion of food using the standard default values as given by the U.S. EPA (Table 33).

Table 33: Calculation of ADI values as exposure dose (US.EPA, 2005)

Exposure factor (EF) =

$$\frac{\text{Frequency of exposure (days year}^{-1}\text{) x exposure duration (years)}}{\text{Exposure duration x 365 days/year}}$$

Exposure Dose (D) =

$$\frac{\text{Contaminant concentration (mg g}^{-1}\text{) x consumption rate (g day}^{-1}\text{) x EF}}{\text{Body weight (kg)}}$$

Body weight (BW): 70 kg – adult

Exposure duration (ED): 9 years – time spend at one residence

*To calculate these values the consumption of two chicken eggs per week was assumed providing an EF factor of 0.28

According to the TDIs listed in Van Oostdam *et al*. (1999), chicken eggs would contribute less than 1% to the TDI of all OCPs analysed (Table 35). The risk of increased exposure to OCPs is thus less than the nutritional benefit derived from eating backyard chicken eggs.

The exposure assessment for DLCs in backyard chicken eggs was calculated differently due to a maximum limit of 4 pgTEQ g^{-1} lm according to EU regulation 1881/2006 (2003). According to this regulation, eggs in the Vanderbijlpark had twice the maximum allowed level (Table 27). Therefore the TDI of these two sites were then determined. According to this calculation, backyard chicken eggs would contribute 18% to the TDI

(2 pg TEQ kg^{-1} day^{-1}) for dioxins (Kijlstra, 2004). Thus, although the exposure associated with the consumption of backyard chicken eggs for OCPs is negligible, the high level of DLCs is cause for concern.

Table 34: ADI values ($mg.g^{-1}.day^{-1}$) for OCs in chicken eggs from South Africa

Area	ΣPBDEs	HBCD	HCB	β-HCH	ΣChlordanes	ΣDDT	Mirex
Cbr1*	1.1 x10^{-08}	6.3 x10^{-10}	6.8 x10^{-09}	3.1 x10^{-09}	3.5 x10^{-09}	1.9 x10^{-09}	5 x10^{-10}
Cbr2*	4.8 x10^{-08}	1.4 x10^{-07}	2.6 x10^{-08}	1.1 x10^{-08}	6.7 x10^{-09}	2.9 x10^{-08}	4.3 x10^{-10}
Tsh*	7.6 x10^{-08}	6.9 x10^{-07}	4.7 x10^{-07}	1.2 x10^{-08}	2.6 x10^{-07}	2.1 x10^{-07}	4.6 x10^{-10}
Shv*	2 x10^{-07}	2.2 x10^{-08}	6.3 x10^{-08}	9.1 x10^{-09}	1.8 x10^{-08}	1.8 x10^{-07}	4.2 x10^{-10}
Bfn#	1 x10^{-08}	5.9 x10^{-10}	1.3 x10^{-08}	1.8 x10^{-08}	6.6 x10^{-09}	7.6 x10^{-09}	1.1 x10^{-08}
Pta#	1.2 x10^{-08}	7.1 x10^{-10}	9.3 x10^{-09}	1.6 x10^{-08}	1.1 x10^{-08}	2.6 x10^{-09}	5.7 x10^{-10}
Stb#	1.1 x10^{-08}	6.5 x10^{-10}	1.5 x10^{-08}	2.7 x10^{-08}	1.4 x10^{-09}	1.6 x10^{-08}	5.2 x10^{-10}

TDIs (mg kg^{-1} BW day^{-1}) for Canada as listed in Oostdam *et al*. 1999: HCB = 2.7 x10^{-4}; ΣChlordanes = 5 x10^{-5}; ΣDDT = 0.02; Mirex = 7 x10^{-5}; *Backyard chicken eggs; #Commercial chicken eggs

6 Conclusions and recommendations

POPs have different intrinsic physiochemical properties that dictate their individual environmental behaviour, including bio-accumulation (Lohmann *et al*., 2007). In this regard, the accumulation of POPs in many avian species is well documented. As such, birds' eggs are frequently used as indicators of environmental contamination. Using vertebrates as bioindicaters provides a critical link to the potential ecosystem problems, human exposure, and possible risks (Keithmaleesatti *et al*., 2007).

This study contributed to identifying the current levels of POPs in industrialised areas of South Africa, using wild bird eggs as a bioindicators. Since recent data concerning POPs is scarce in Southern Africa, the current study addresses a serious data gap in recent knowledge of organohalogenated compounds. Further studies are needed, with larger sampling sizes to facilitate statistical analysis. However, the dispersal of bird nesting sites and the number of pairs per site will be a limiting factor. The lack of guideline values for birds in South Africa is hampering in determining potential toxicity. However, very little is known about the direct relationship between levels and concentration in temperate, Southern-hemisphere birds. This is an area where further research is crucial to the continued viability of avian populations.

As found in other similar studies, there were significant differences in the concentrations of organohalogen compounds between different bird species. These differences could be attributed to multiple factors, including the area of sampling, diet, and feeding habitat of the individual species, as well as their proximity to anthropogenic activity. For OCPs and PCBs, concentrations were related to the position of the species in the food web. However, for BFRs the association to human activity had a significant effect. Levels of OCPs and BFRs were higher in the White-breasted Cormorant, the top predator species in this study whereas levels of BFRs were higher in the Sacred Ibis.

6.1. BFRs in the wild bird eggs

BFRs were present in all samples analysed with congener profiles, showing a shift toward BFRs that are not regulated.

New findings

- In the current study area, the levels of BFRs are dictated by the exposure of wild birds to BFRs through contact with humans or human waste.

- Association with humans have a stronger influence on BFR levels than trophic level as indicated by the high levels found in lower trophic level bird in close association compared to higher trophic bird species
- The congener pattern differences between terrestrial and aquatic species appeared different to those reported in literature. This possibly indicates differences in main sources of BFRs. However, differences in diet, feeding habitats and sampled species could have also contributed to this finding.
- Levels measured in eggs from the current study area were below the NOEL, as obtained from literature. Therefore, BFR residues probably do not pose a threat to the birds in the specific area studied.

Recommendations

- There are no restrictions on importing or marketing PBDE-containing products in South Africa, therefore, BFR exposure in wild bird populations will escalate. This necessitates ongoing monitoring to assess time-trends and contamination sources of BFRs. The addition of commercial PBDEs to the SC may, however, eventually result in reductions of PBDEs, but PBDE leakage from wastes will likely be a long-term aspects that needs monitoring.
- Monitoring programs should include both HBCD as well as the higher brominated PBDEs due to the shift in use of technical products. However, HBCD has been introduced in the SC process, and may become a banned or restricted chemical in the years to come.programs should include both HBCD as well as the higher brominated PBDEs due to the shift in use of technical products. However, HBCD has been introduded in the SC process, and may become a banned or restricted chemical in the years to come.
- The differences between birds with different feeding habitats should be explored further to elucidate possible differences in the behaviour of BFRs in the South African environment. To exclude influence of diet, a larger group of species with a wider range in niches, especially including those associated with the terrestrial environment, should be studied.
- Although levels were below NOELs, there is no literature studying the effects of BFRs on South African birds of birds from comparable regions. Due to unique exposure profiles and life history characteristics, the effects of BFRs on avian health could be underestimated. Further, toxicologically relevant research is needed with regards to BFRs.

6.2. OCPs in wild bird eggs

As seen in previous studies, ΣOCPs were present in all samples analysed. An increase in concentration corresponded to the trophic level, with *p,p*'-DDE being the predominant congener in most species.

New findings

- The exposure profile for the Crowned Lapwing was different to all other species. This was found in other international studies, but the reason has not been clarified. The Crowned Lapwing had the highest level of chlordanes and HCB. This could be due to their diet, consisting mainly of termites, or due to a unique metabolism associated with its taxon.
- Levels of *p,p'*-DDT and mirex are likely due to long-range or atmospheric transport.
- Comparisons with other published studies indicate relatively high concentrations of OCPs in the wild bird eggs studies. South Africa has a unique exposure to OCPs since HCH, DDT and chlordane (until very recently) are actively used within the country's borders, as indicated by the high level of OCPs when compared to other studies from literature.
- Levels of *p,p*-DDT indicate recent environmental exposure. However, low background levels show that the exposure is likely due to atmospheric transport from the northern and/or eastern regions of the country where DDT is actively applied in IRS programs.
- There was no correlation between the presence of DDE and eggshell thinning. Eggshell thinning due to the presence of DDD, however, could affect granivore species.

Recommendations

- The atmospheric transport of DDT, in combination with environmental levels should be investigated. This can be achieved through the modelling of air currents, considering meteorological conditions with models such as the hybrid single particle lagrangian integrated trajectory model (HYSPLIT).
- Due to deregistration of chlordane and the addition of α-, β-, & γ-HCH in the SC, the congener profiles of OCPs will change. Therefore, further monitoring of these chemicals will increase our understanding of the properties of these chemicals in the South African environment.

- To assess the effect of habitat on the concentration of organohalogens, a broader set of both aquatic and terrestrial species will have to be included so that each trophic guild is represented for each major feeding habitat.
- Absence of conclusive eggshell thinning does not exclude other negative health effects, since levels of DDE in piscivore species were approaching toxicological thresholds. Therefore, the effect of eggshell thinning in granivores need to be assessed. To achieve this, a larger number of eggs need to be collected per species.
- Studies that include granivores are also important due to their close associations with humans.
- Sub-lethal effects such as endocrine disruption need further investigation.

6.3. PCBs in wild bird eggs

As found in other studies, the predominant PCB congeners were CB-130, -180 and -153. All species had quantifiable levels of PCBs, with 88% of the congeners measured being found in more than 80% of samples. As in the case of OCPs, PCB levels were highest in piscivore species. Piscivore species also had the highest percentage of DL-PCBs - once again indicating increased potential health risks from organohalogen compounds.

- Metabolic group I PCBs were more prevalent, likely due to resistance to degradation.
- There were low levels of metabolic group IV and V, which are more biodegradable.
- Higher chlorinated congeners were more prevalent in higher trophic levels while lower chlorinated congeners were associated with lower trophic levels.
- The PB-type inducer PCBs were more prevalent than mixed-type PCBs, indicating a prevalence of less toxic PCBs in wild bird eggs.

Recommendations

- The high prevalence of CB-138 and -180 make them ideal monitoring PCBs. However, due to the toxicological importance of PCB-153, it should also be included in residue monitoring programmes
- 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 DLC in wild bird eggs.

- DLCs have been linked to detrimental health effects and the presence of PCB 105, 114, and other DL-PCBs indicate that there could be measurable levels of other DLCs in wild bird populations. Further study in this regard is needed.

6.4 Organohalogenated compounds in wild and domesticated bird eggs

Findings

- As expected, PCA analysis separated organohalogenated compounds according to the physiochemical properties of the specific congener, as well as seperating the bird species according to their biological traits and feeding habitats.
- These results confirm observations from known interactions between the biology of bird species and the chemical properties of organohalogenated compounds.
- Results confirmed the usefulness of PCAs in ecotoxicological studies.
- Ratios of DDE / PCBs and PBDEs / PCBs elucidated site specific differences in exposure of the various organohalogens as well as differences in exposure due to biological factors.

Important aspects for further consideration/research

Toxicological impacts on wild birds

It is difficult to assess the toxicological potential and impact of various mixtures of halogenated organic compounds. Although the individual groups were all below NOELs, this does not mean that there are no negative effects associated with current levels of organohalogens. Interactions between PBDEs and other environmental pollutants such as PCBs can be responsible for detrimental health effects. As stated by Jaspers *et al*. (2005), “even low chronic exposure in combination with other non-anthropogenic stressors may lead to reduced productivity.” These interactions and there possible effects on the integrity of wild bird populations by impacting fertility, reproductive success, and behaviour cannot be quantifiably determined. The fact that the Ratcliffe index remained approximately the same, decades after DDT use was banned shows that increase in other chemicals such as PBDEs and current use pesticides could have replaced the effects of DDT to a similar degree in contemporary bird eggs. The banning of DDT and its eventual reduction in the environment likely overlapped with the increased release into the environment of PBDEs and other compounds studied here, while some compounds (notably chlordane and lindane) have

been used on a large scale up to now. However, these effects are likely integrated as mixtures and may not be as easily attributed to a single compound as in the case of DDT's effect on eggshell thinning.

South African bird populations have unique exposure profiles as discussed in Chapter 2, section 2.5. Many species that are migratory in other regions of the world are non-migratory here. The exposure of non-migratory birds to POPs changes to a lesser degree when compared with changes in diet and habitat associated with migratory behaviour. However, very few migratory birds breed in southern Africa. Compared with species in colder areas, species from moderate and tropical climates also tend to be longer-lived and lay smaller clutches, with extended parental care. Another phenomenon is that most organohalogens compounds including PBDEs and PCB concentrations are higher in the female compared to her eggs. Thus, although concentrations in eggs are lower than risk threshold, the pollutant profiles in adult birds could be at levels where negative health effects and possible behavioural changes may be expected. Even if these thresholds are not reached, the additional physiological stress placed on individuals in combination with habitat destruction, food shortages, and a changing climate could seriously impact wild bird populations in South Africa.

DLC in food sources

Levels of dioxins in backyard chicken eggs that are above the EU recommended limits are cause for concern. These chemicals are highly toxic and can lead to myriad of health effects. In low-income populations often impacted by HIV and malnutrition, these effects could be further magnified. Eggs are not the only backyard produce utilised. Often low-income families will have small vegetable gardens and goats that are used to obtain milk and, in some cases, meat. These families also rely on alternative fuel sources such as wood fires and kerosene. These factors can all increase dioxin exposure making further research in this regard crucial. Although OCP levels in backyard chicken eggs are below levels of concern in the current study area, this profile can differ in more agriculturally impacted areas. In the northern parts of South Africa where DDT is still actively applied to huts, backyard poultry occurs frequently. In these areas poultry is not only important for food security but also for cultural and religious considerations (Swatson *et al*., 2009). Considering that DDT is directly applied to huts and that the soil surrounding the huts will have high levels of DDT, foraging chickens will correspondingly have increased levels. More knowledge on the exposure profiles of these communities through the consumption of local produce is essential.

In conclusion, measureable levels of all the compounds analysed indicate an environment impacted by anthropogenic activity that now and in the long term could negatively affect both the environment and human health. The ongoing monitoring of bird eggs is advisable as it provides essential information on time-trends and changing exposure profiles to organohalogenated compounds, while minimally impacting wild bird populations. However, studies determining levels in adult birds as well as prey species such as fish could provide invaluable insight into the accumulation of organohalogens in the South African environment. Additionally, the findings indicate that the dietary human intake of organohalogen compounds of low-income communities have to be assessed.

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Supplementary data

Table S1: Concentrations (ng g^{-1} ww) of BFRs in wild bird eggs collected in Gauteng and Free State Provinces of South Africa

Sample nr	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-206	BDE-207	BDE-208	BDE-209	ΣPBDEs	HBCD	ΣBFR
Detection limit	0.02	0.04	0.02	0.02	0.02	0.02	0.04	0.11	0.05	0.02	3.12	NA	0.29	NA
Relative recovery (%)	103	105	117	101	101	94	105	112	113	115	230	NA	103	NA
SIs11	ND	0.128	0.482	0.198	4.688	0.344	15.352	0.864	5.058	2.005	14.584	43.704	3.033	46.737
SIs12	ND	0.140	0.568	ND	5.235	0.447	15.038	ND	0.216	0.200	15.049	36.892	4.527	41.419
SIs13	ND	0.223	0.790	0.345	6.950	0.619	16.565	1.054	5.796	2.053	21.142	55.536	3.154	58.690
SIs14	ND	0.294	1.349	0.475	6.054	0.668	16.392	0.906	3.900	1.577	12.980	44.595	3.371	47.966
SIs15	ND	0.137	0.465	0.211	4.749	0.396	12.183	0.942	5.252	1.804	20.175	46.314	1.588	47.902
SIs16	ND	0.145	0.441	0.120	3.794	0.329	11.230	1.019	4.500	1.830	19.867	43.273	1.830	45.103
SIs17	ND	0.132	0.422	0.251	3.861	0.417	14.829	0.551	6.139	1.073	9.576	37.251	2.473	39.724
SIs18	ND	0.110	0.595	0.214	50.620	4.509	121.180	0.902	15.742	1.645	25.403	220.920	1.310	222.230
SIs19	ND	0.178	0.410	ND	2.459	0.206	6.656	1.226	4.308	1.911	17.607	34.960	1.433	36.393
SIs20	ND	0.116	0.248	0.078	0.374	0.068	0.432	0.113	0.288	0.258	ND	1.975	0.922	2.897
SIs21	ND	0.200	0.687	0.318	9.166	0.780	29.841	0.846	4.926	1.864	16.222	64.850	1.263	66.113
SIs22	ND	0.360	1.116	0.332	4.760	0.412	10.602	0.570	2.954	1.176	10.562	32.844	4.835	37.678
SIs23	ND	0.591	1.764	0.724	2.354	0.323	5.182	0.369	1.436	0.473	8.839	22.055	2.031	24.085
SIs24	ND	0.106	0.402	0.298	3.931	0.550	12.011	1.794	5.517	1.466	25.477	51.553	3.439	54.992
SIs25	ND	0.523	1.483	0.673	3.612	0.590	7.393	0.942	3.663	1.387	24.133	44.400	3.783	48.183
SIs26	ND	0.325	1.099	0.338	3.558	0.295	7.828	0.414	2.048	0.872	7.551	24.328	4.206	28.533
CEs27	ND	0.827	1.939	0.391	1.021	1.613	0.783	ND	0.205	0.128	ND	6.908	1.168	8.076
CEs28	ND	0.048	0.108	0.072	0.657	0.103	0.409	ND	0.062	0.030	ND	1.489	ND	1.489
CEp29	ND	ND	ND	ND	0.186	ND	0.217	ND	ND	ND	ND	0.403	ND	0.403
CEp30	ND	ND	7.736	ND	0.031	0.054	0.080	ND	ND	ND	ND	7.901	ND	7.901
CEsb31	ND	ND	7.630	ND	0.031	0.048	ND	ND	ND	0.024	ND	7.733	ND	7.733
CEsb32	ND	ND	ND	ND	0.053	ND	0.053	0.165	ND	0.026	ND	0.298	ND	0.298
ADk33	ND	0.383	ND	0.394	0.216	0.778	0.058	ND	ND	0.033	ND	1.862	0.615	2.477
ADk34	ND	0.378	0.088	3.277	0.838	2.381	0.381	ND	0.050	ND	ND	7.392	0.736	8.129
ADp35	ND	0.154	ND	0.264	0.434	0.617	0.115	0.170	ND	ND	ND	1.753	0.568	2.321
ADp36	ND	0.387	ND	0.497	0.325	0.785	0.040	ND	ND	ND	ND	2.033	0.442	2.474
ADp37	ND	0.060	ND	0.075	0.060	0.142	ND	ND	0.022	ND	ND	0.360	ND	0.360
ADp38	ND	0.203	ND	0.451	0.535	0.843	0.164	0.349	0.052	ND	ND	2.597	0.563	3.159
ADp39	ND	0.139	9.669	0.409	0.356	0.682	0.069	ND	ND	ND	ND	11.324	0.425	11.749
ADk40	ND	2.014	0.083	4.679	2.489	6.065	0.995	0.907	0.228	0.130	ND	17.592	6.435	24.027
ADk41	ND	0.090	9.735	0.101	0.047	0.228	ND	ND	ND	ND	ND	10.202	ND	10.202

Sample nr	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-206	BDE-207	BDE-208	BDE-209	ΣPBDEs	HBCD	ΣBFR
ADk42	ND	0.110	ND	0.076	0.104	0.439	0.151	ND	0.069	ND	ND	0.949	ND	0.949
ADk43	0.179	6.477	ND	3.793	0.925	3.592	0.644	0.901	0.226	ND	3.160	19.897	9.595	29.493
ADk44	0.108	4.182	ND	1.783	0.353	1.544	0.146	0.284	0.060	ND	ND	8.461	1.494	9.954
ADk45	ND	0.402	ND	0.288	0.069	0.806	0.185	ND	ND	ND	ND	1.751	ND	1.751
WCr7	ND	0.417	ND	0.430	0.457	0.681	0.118	0.407	0.108	ND	ND	2.618	0.314	2.932
WCr8	ND	1.391	0.119	1.082	1.597	2.114	0.091	ND	ND	ND	ND	6.393	0.965	7.358
WCr9	ND	0.981	0.086	0.846	1.219	1.561	0.148	ND	ND	ND	ND	4.841	0.822	5.663
WCr10	ND	0.630	0.083	0.463	0.566	0.818	0.066	ND	ND	ND	3.188	5.814	0.399	6.213
BCHs5	ND	0.056	0.096	0.061	0.179	0.049	0.087	ND	ND	ND	ND	0.529	ND	0.529
BCHs6	ND	0.052	0.113	ND	0.208	0.047	0.159	0.349	0.087	ND	ND	1.015	ND	1.015
BHHk4	ND	ND	0.062	ND	1.914	0.151	2.488	0.553	0.640	0.415	16.844	23.067	0.606	23.673
B/GHp1	ND	ND	0.181	0.110	0.522	0.185	0.634	ND	ND	ND	ND	1.631	ND	1.631
B/GHp2	ND	0.044	0.298	0.215	22.208	0.668	24.738	1.622	0.409	0.242	ND	50.445	0.415	50.860
B/GHp3	ND	0.056	0.366	0.186	7.699	0.323	11.741	ND	0.361	0.173	ND	20.905	ND	20.905
CPvp53	ND	1.288	1.295	0.395	1.000	0.368	1.224	0.900	0.222	0.137	ND	6.829	ND	6.829
CPvp54	ND	0.679	0.947	0.311	0.237	0.139	0.209	ND	0.070	0.044	ND	2.636	ND	2.636
CPvp55	ND	0.223	0.313	0.112	0.154	0.100	0.109	1.332	0.340	0.207	ND	2.889	ND	2.889
CPvp56	ND	0.976	0.393	0.060	0.259	0.092	0.381	0.299	0.075	ND	ND	2.535	0.398	2.933
CPsb57	ND	0.369	0.384	0.092	0.179	0.063	0.166	ND	0.100	0.055	ND	1.408	0.319	1.726
CPsb58	ND	0.541	0.460	ND	0.231	0.088	0.207	ND	0.111	0.042	ND	1.680	ND	1.680
RCs46	ND	0.134	8.136	ND	0.071	0.082	0.055	ND	ND	ND	ND	8.478	1.108	9.585
RCs47	ND	0.214	9.692	ND	0.034	0.077	0.046	3.255	1.284	0.563	193.250	208.413	0.358	208.772
RCk48	ND	2.636	1.085	0.197	0.216	0.091	ND	ND	0.111	0.098	ND	4.433	0.383	4.817
RCk49	ND	2.974	1.204	0.480	0.260	0.091	ND	0.412	0.087	0.070	ND	5.579	0.323	5.902
RCvp50	ND	2.827	1.243	0.330	0.170	0.095	0.203	0.181	0.203	0.201	ND	5.453	2.242	7.695
RCvp51	ND	0.089	9.802	ND	0.080	0.072	0.043	ND	0.067	ND	ND	10.154	ND	10.154
CDsb52	ND	ND	10.985	0.033	0.076	0.067	ND	ND	0.052	ND	ND	11.214	0.343	11.556
Ssb64	ND	0.027	10.584	ND	0.167	ND	0.172	ND	0.131	0.072	ND	11.154	0.373	11.527
Ssb65	ND	0.057	11.440	ND	0.090	0.080	0.139	ND	0.057	ND	ND	11.863	0.388	12.251
Svp66	ND	31.618	10.161	ND	0.206	0.086	0.152	ND	0.115	0.100	ND	42.438	0.360	42.798
SMWp60	ND	0.542	10.368	0.137	0.277	0.089	0.190	ND	0.222	0.144	3.857	15.825	0.368	16.193
SMWvp61	ND	147.175	0.548	0.090	0.145	0.029	0.073	ND	0.081	0.055	ND	148.197	0.357	148.554
SMWvp62	ND	11.242	15.880	0.052	2.277	1.432	1.766	0.318	0.633	0.380	ND	33.981	0.334	34.315
SMWp63	ND	0.391	0.488	0.079	0.345	0.071	0.245	ND	0.152	0.073	ND	1.843	1.284	3.126

Table S2: Factor loadings for the PCA analysis of all BFRs in South African wild bird eggs.

EIG NAME	*0.3733* AX1	*EIG* NAME	*0.3179* AX2	*EIG* NAME	*0.092* AX3	*EIG* NAME	*0.0838* AX4
SIs18	-1.1239	ADk43	-0.9239	ADp39	-0.667	SMWvp62	-0.6729
SIs19	-1.0891	ADp38	-0.745	B/GHp1	-0.5855	B/GHp3	-0.541
BHHk4	-0.9476	ADk40	-0.7346	CDsb52	-0.5545	CEs27	-0.4972
SIs11	-0.9204	SIs18	-0.7335	CEp30	-0.5041	Svp66	-0.4758
SIs16	-0.919	ADk44	-0.6874	ADk41	-0.4725	SMWvp61	-0.3896
SIs15	-0.8911	WCr7	-0.6848	Ssb65	-0.4264	SIs18	-0.3128
SIs21	-0.8781	ADp35	-0.661	ADk34	-0.4089	WCr8	-0.3035
SIs24	-0.843	B/GHp2	-0.6608	B/GHp2	-0.3784	ADk34	-0.2876
SIs17	-0.8288	ADp36	-0.581	CEsb3	-0.37	B/GHp2	-0.2713
SIs13	-0.8284	ADk33	-0.564	B/GHp3	-0.3454	WCr9	-0.2681
SIs12	-0.7177	ADk45	-0.5517	RCs46	-0.3013	CPvp53	-0.2554
SIs14	-0.712	ADk34	-0.5432	RCvp51	-0.2916	CPvp54	-0.2517
B/GHp2	-0.7047	ADk42	-0.5108	SIs18	-0.2231	SIs23	-0.2463
SIs22	-0.6433	SIs24	-0.4475	WCr8	-0.2199	SIs12	-0.2442
B/GHp3	-0.5927	SIs21	-0.4414	SIs12	-0.2162	ADp39	-0.2348
SIs26	-0.5791	B/GHp3	-0.4248	WCr9	-0.216	SMWp60	-0.2246
Ssb64	-0.5629	SIs17	-0.4222	CEs28	-0.2072	ADk40	-0.2204
SIs25	-0.5412	BHHk4	-0.4161	Ssb64	-0.1798	SIs26	-0.1986
RCs47	-0.5249	SIs11	-0.3869	BCHs5	-0.1718	SIs21	-0.1907
SIs23	-0.3454	WCr9	-0.3821	WCr10	-0.1532	SIs14	-0.1846
SIs20	-0.2924	SIs15	-0.3789	CEs27	-0.1464	SIs22	-0.182
CEp29	-0.1919	SIs13	-0.3632	ADp35	-0.1367	RCvp50	-0.1366
BCHs6	-0.1372	SIs16	-0.3304	ADp38	-0.1004	RCk48	-0.1338
CEsb32	-0.136	WCr8	-0.3257	BCHs6	-0.0364	SMWp63	-0.1027
CEs28	-0.0978	CEp29	-0.3243	ADp36	-0.0325	SIs17	-0.081
CEp30	-0.0941	ADp37	-0.3066	ADk42	-0.0275	SIs25	-0.0795
Ssb65	-0.0934	SIs14	-0.2741	ADp37	-0.0084	CPsb58	-0.0755
CPvd55	-0.0773	WCr10	-0.2315	CEp29	0.0029	SIs13	-0.0718
CEsb31	-0.0587	SIs22	-0.2199	BHHk4	0.0154	CPsb57	-0.052
SMWvp62	-0.0526	CEs28	-0.1912	ADk45	0.0204	WCr10	-0.0453
CPsb58	-0.0417	B/GHp1	-0.1885	SIs21	0.031	B/GHp1	-0.0246
SMWp60	-0.0229	SIs26	-0.1742	ADk40	0.0324	CEs28	-0.0176
B/GHp1	0.008	CEsb32	-0.1718	SIs14	0.0371	SIs11	-0.0059
RCvp51	0.0279	SIs25	-0.1679	SMWp60	0.0402	CPv56	0.0044
SMWp63	0.0364	SIs12	-0.1634	CPvp54	0.0411	Ssb65	0.0247
CPvp53	0.0638	SIs19	-0.1234	SIs24	0.0563	RCk49	0.0261
CDsb52	0.0772	SIs23	-0.0578	SIs23	0.0643	SIs15	0.0285
CPsb57	0.1254	SIs20	0.0001	SIs17	0.0683	SIs20	0.0422
RCs46	0.174	BCHs5	0.0067	WCr7	0.082	Ssb64	0.0543
BCHs5	0.2067	BCHs6	0.0562	SIs26	0.0837	ADk41	0.0592
CEs27	0.207	CPvp53	0.0792	SIs13	0.0889	RCvp51	0.0723
CPv56	0.2852	CEs27	0.1105	ADk33	0.0893	SIs16	0.0772
Svp66	0.2872	CPvd55	0.1481	SIs22	0.1001	RCs46	0.0804
RCvp50	0.2957	CPv56	0.1671	SIs11	0.1159	ADk45	0.0956
CPvp54	0.3266	SMWp63	0.2001	ADk43	0.1185	ADp36	0.1106
ADk42	0.3629	CPsb57	0.2282	SIs15	0.1192	ADk33	0.1166
ADk41	0.4651	CPvp54	0.2862	CPv56	0.1307	SIs24	0.1618
ADp37	0.5131	CPsb58	0.3561	SIs25	0.1335	BCHs5	0.1927
RCk48	0.5302	RCvp50	0.3726	CPvp53	0.1454	CEp30	0.2034
ADp39	0.5435	RCk49	0.4859	SMWp63	0.1522	CDsb52	0.2122
ADp38	0.591	RCk48	0.5425	SIs20	0.1667	ADk44	0.2152
ADp35	0.5986	SMWvp61	0.5754	CPsb57	0.1683	SIs19	0.2189
ADk40	0.6054	SMWvp62	0.5755	ADk44	0.1688	ADp35	0.2333
WCr7	0.619	ADp39	0.6066	SIs16	0.1712	ADk42	0.235
RCk49	0.6343	SMWp60	0.7705	CEsb32	0.2106	ADp38	0.2387
ADk45	0.7206	ADk41	0.923	CPsb58	0.2413	WCr7	0.2403
ADk33	0.7251	Ssb65	0.9356	SMWvp62	0.3107	ADk43	0.2681
ADk34	0.7833	Ssb64	0.9509	CPvd55	0.3403	CEsb31	0.2981
SMWvp61	0.7868	CEp30	0.9609	SIs19	0.3719	CPvd55	0.3439
WCr10	0.7995	RCs46	0.9695	RCk49	0.4065	BCHs6	0.4091
WCr9	0.8676	CDsb52	0.972	RCs47	0.4088	CEp29	0.4112
ADp36	0.8728	RCvp51	1.0127	RCk48	0.4233	BHHk4	0.4451
WCr8	0.9745	Svp66	1.0595	RCvp50	0.4512	ADp37	0.4654
ADk43	1.1569	CEsb31	1.0604	Svp66	0.6869	CEsb32	0.7064
ADk44	1.2177	RCs47	1.0836	SMWvp61	1.0859	RCs47	0.9886

Table S3: Factor scores for the PCA analysis of all BFRs in South African wild bird eggs.

EIG	***0.3733***		***0.3179***		***0.092***		***0.0838***
NAME	**AX1**	**NAME**	**AX2**	**NAME**	**AX3**	**NAME**	**AX4**
BDE-207	-1.4325	BDE-153	-1.1892	BDE-154	-1.3768	BDE-153	-1.4639
BDE-208	-1.36	BDE-183	-0.8826	BDE-153	-0.8355	BDE-183	-0.9801
BDE-183	-1.2418	BDE-154	-0.8116	BDE-28	-0.6981	BDE-47	-0.8235
BDE-153	-0.603	BDE-100	-0.7718	BDE-99	-0.6669	BDE-99	-0.5172
BDE-209	-0.4966	HBCD	-0.2688	BDE-100	-0.5125	BDE-100	-0.4247
BDE-206	-0.238	BDE-206	-0.2578	BDE-183	-0.372	BDE-154	-0.354
BDE-99	-0.1996	BDE-207	-0.1901	HBCD	-0.2612	BDE-208	-0.1472
HBCD	0.4717	BDE-208	0.0971	BDE-209	-0.1954	BDE-207	0.2462
BDE-154	1.0527	BDE-209	0.3358	BDE-206	0.5871	HBCD	0.6938
BDE-28	1.0661	BDE-47	0.3693	BDE-207	0.9977	BDE-28	1.6344
BDE-100	1.2433	BDE-28	0.3816	BDE-208	1.3702	BDE-206	2.537
BDE-47	1.2607	BDE-99	1.6993	BDE-47	1.7484	BDE-209	2.54

Table S4: Concentrations (ng g^{-1} ww) of OCPs in wild bird eggs collected in Gauteng and Free State Provinces of South Africa

Sample nr	HCB	α-HCH	β-HCH	γ-HCH	Oxy-chlordane	Trans chlordane	cis-chlordane	Trans nonachlor	cis-nonahlor	p,p'-DDE	p,p'-DDD	p,p'-DDT	Mirex	ΣOCPs
Detection limit	0.011	0.008	0.021	0.011	0.018	0.017	0.017	0.018	0.017	0.012	0.020	0.023	0.090	
Relative recovery (%)	90-112	56-85	62-88	76-100	80-91	82-112	80-109	88-118	90-125	72-94	65-92	99-117	84-91	
SIs11	0.7	0.009	0.986	0.212	2.500	0.026	0.065	3.465	0.189	42.829	0.204	4.330	0.110	55.666
SIs12	0.8	0.020	1.169	0.225	3.065	ND	0.061	4.637	0.227	55.684	0.175	5.110	ND	71.213
SIs13	1.0	0.011	1.634	0.376	1.338	0.042	0.108	1.957	0.142	90.639	2.880	26.952	0.146	127.205
SIs14	1.3	0.018	1.207	1.647	2.244	0.035	0.118	2.834	0.160	26.655	0.241	6.670	0.179	43.340
SIs15	0.7	0.010	1.208	0.415	0.918	0.036	0.069	1.327	0.102	55.839	2.717	16.394	0.102	79.811
SIs16	0.7	0.010	0.766	0.221	1.308	0.042	0.065	1.249	0.079	35.700	0.240	8.256	0.100	48.708
SIs17	1.3	0.009	0.712	0.204	0.624	0.018	0.067	0.937	0.076	16.680	0.166	4.438	0.137	25.356
SIs18	1.3	0.008	1.016	0.502	1.429	ND	0.026	1.038	0.064	31.105	0.159	6.806	0.113	43.540
SIs19	2.6	0.023	0.925	0.477	2.261	0.033	0.071	1.230	0.075	30.697	0.278	10.325	0.112	49.064
SIs20	0.4	ND	0.328	0.075	0.514	0.027	0.041	0.437	0.040	3.866	0.139	1.171	ND	7.009
SIs21	1.0	ND	1.622	0.299	1.848	0.043	0.112	3.391	0.295	51.221	0.260	7.592	0.108	67.790
SIs22	2.2	0.012	1.264	0.652	1.629	0.036	0.085	1.467	0.106	66.679	0.426	15.084	0.113	89.720
SIs23	1.8	0.022	1.601	1.520	1.739	0.029	0.119	1.605	0.133	26.144	0.359	8.683	0.125	43.882
SIs24	0.5	0.022	0.532	0.284	1.273	0.021	0.088	0.699	0.028	28.807	0.138	3.449	0.140	36.010
SIs25	0.8	0.018	1.046	0.944	2.303	2.381	0.209	2.381	0.141	48.833	0.429	9.650	0.153	69.312
SIs26	0.5	0.018	3.453	1.622	1.098	0.027	0.110	1.378	0.134	22.586	0.220	1.929	ND	33.064
CEs27	0.6	ND	1.292	0.037	0.708	0.022	0.038	0.568	0.093	51.638	0.092	1.857	0.220	57.156
CEs28	0.3	0.020	0.190	0.020	0.509	ND	0.031	0.880	0.140	31.627	0.028	0.711	0.376	34.842
CEp29	0.7	0.021	0.676	ND	0.097	ND	ND	0.099	0.030	7.374	0.040	0.384	0.229	9.622
CEp30	1.8	0.022	0.077	0.018	0.110	ND	ND	0.051	0.026	8.663	0.030	0.219	0.733	11.707
CEsb31	0.3	0.030	0.043	0.041	0.035	ND	ND	0.023	0.035	4.721	0.027	0.144	1.738	7.130
CEsb32	0.3	0.020	0.091	ND	0.093	ND	ND	0.070	0.036	2.721	0.023	0.175	0.563	4.117
ADk33	2.5	0.100	49.205	0.092	3.021	0.035	0.172	0.247	0.486	2441.623	4.217	146.108	0.318	2648.141
ADk34	2.9	0.095	4.017	0.098	2.449	0.018	0.177	0.264	0.680	462.540	0.933	6.158	1.339	481.690
ADp35	2.3	0.063	9.277	2.371	2.672	ND	0.205	0.102	0.547	122.114	0.110	0.604	0.954	141.365
ADp36	5.4	0.080	10.372	2.886	11.892	ND	0.407	0.333	1.028	177.774	0.244	1.686	0.848	212.927
ADp37	0.9	0.058	2.583	0.942	0.796	0.030	0.020	0.042	0.203	133.789	0.048	0.480	0.788	140.709
ADp38	5.71	0.165	23.624	4.348	8.036	0.078	0.288	0.177	1.242	292.688	0.303	1.397	0.948	339.000
ADp39	5.14	0.114	17.750	2.175	8.467	0.287	0.255	0.280	1.397	222.109	0.202	1.355	0.919	260.452
ADk40	6.04	0.145	7.877	6.357	5.165	0.091	0.854	0.448	3.418	391.696	0.852	3.575	0.894	427.415
ADk41	0.99	0.067	1.438	0.213	0.626	0.273	0.051	0.787	0.301	146.558	0.522	0.651	0.490	152.971
ADk42	0.70	0.027	1.189	0.060	1.212	0.054	0.084	0.097	0.110	225.125	0.487	0.702	0.174	230.026
ADk43	8.57	10.591	135.874	9.477	25.812	0.609	3.632	8.868	3.663	932.387	6.821	2.033	0.384	1148.718
ADk44	2.33	0.123	3.251	2.985	2.553	0.128	0.445	0.526	0.732	602.795	2.686	1.659	ND	620.218
ADk45	0.69	1.470	138.659	0.368	3.035	0.027	0.140	0.276	0.863	652.077	1.344	1.134	ND	800.082
WCr7	0.79	0.089	7.503	2.920	3.468	0.043	0.120	0.282	0.532	471.553	0.299	0.365	0.114	488.077
WCr8	2.48	0.216	12.965	6.375	8.685	0.113	0.206	0.418	0.811	723.280	0.496	0.695	0.360	757.103
WCr9	1.75	0.160	9.944	4.415	6.660	ND	0.141	0.300	0.357	422.018	0.343	0.515	0.271	446.870

Table S4 continued: Concentrations (ng g^{-1} ww) of OCPs in wild bird eggs collected in Gauteng and Free State Provinces of South Africa

Sample nr	HCB	α-HCH	β-HCH	γ-HCH	Oxy-chlordane	Trans chlordane	cis-chlordane	transnonachlor	cis-nonahlor	p,p'-DDE	p,p'-DDD	p,p'-DDT	Mirex	ΣOCPs
WCr10	1.25	0.152	15.878	3.099	3.651	0.066	0.149	0.267	0.589	921.550	0.306	0.388	0.181	947.528
BCHs5	0.42	0.009	0.551	0.041	0.546	ND	0.038	0.392	0.087	149.595	15.936	0.116	0.115	167.842
BCHs6	0.46	0.008	0.451	0.107	0.654	ND	0.069	0.526	0.141	194.390	10.837	4.363	0.136	212.143
BHHk4	0.20	0.021	2.195	0.353	1.382	ND	0.098	1.842	0.097	77.784	0.271	0.642	0.139	85.027
B/GHp1	3.06	ND	4.983	0.025	0.974	ND	0.045	0.360	0.045	167.594	0.055	0.289	37.739	215.172
B/GHp2	3.36	ND	9.535	0.028	8.419	0.050	0.499	6.936	0.480	272.983	0.495	14.222	1.470	318.479
B/GHp3	3.68	0.008	4.661	0.025	8.751	ND	0.591	5.578	0.561	45.636	0.116	1.210	3.399	74.221
CPvp53	79.12	0.047	7.337	0.519	10.435	0.237	1.073	73.688	5.656	25.029	0.710	2.643	0.705	207.198
CPvp54	5.10	0.025	0.958	0.511	9.904	0.052	0.482	37.242	4.200	4.916	0.340	1.094	6.300	71.122
CPvp55	4.81	0.013	0.310	0.075	1.185	0.019	0.066	2.957	0.349	0.978	0.206	0.618	0.460	12.044
CPvp56	19.44	0.085	1.263	1.255	27.901	0.370	1.659	93.376	11.096	1.675	0.284	0.444	0.942	159.795
CPsb57	19.22	0.024	0.784	0.201	124.705	0.553	2.491	463.914	55.801	2.133	0.398	1.659	1.014	672.899
CPsb58	19.79	0.030	3.311	0.341	49.576	0.715	3.988	266.142	27.403	6.284	0.462	2.068	4.368	384.482
RCs46	0.53	0.040	1.747	0.052	0.431	0.080	0.093	0.107	0.187	8.713	0.035	0.299	ND	12.314
RCs47	0.49	0.031	1.706	0.030	0.317	0.035	0.092	0.082	0.194	7.584	ND	0.277	ND	10.839
RCk48	0.33	0.036	2.150	0.181	0.359	0.024	0.326	0.259	0.748	59.359	0.059	0.350	ND	64.181
RCk49	0.28	0.010	2.255	0.119	0.515	0.039	0.371	0.250	0.260	67.934	0.023	0.347	ND	72.399
RCvp50	2.17	0.030	1.743	0.325	0.544	0.044	0.272	0.062	0.341	74.994	0.067	0.642	ND	81.234
RCvp51	1.30	0.015	0.369	0.018	0.042	0.045	0.036	ND	0.036	4.379	0.089	0.132	ND	6.457
CDsb52	0.24	0.012	0.061	0.113	0.196	0.050	0.045	0.347	0.037	2.881	0.041	0.162	ND	4.181
Ssb64	1.05	0.030	0.133	0.350	0.116	0.055	0.040	0.068	0.036	2.526	0.088	0.123	ND	4.616
Ssb65	2.56	0.024	0.048	0.189	0.083	0.056	0.052	0.167	0.041	0.552	0.020	0.226	ND	4.022
Svp66	0.63	0.032	0.622	0.455	0.238	0.060	0.082	0.146	0.087	8.962	0.057	0.066	ND	11.435
SMWp60	0.62	0.031	0.736	1.432	0.525	0.064	0.120	0.310	0.242	28.011	0.610	0.615	ND	33.316
SMWvp61	0.50	0.010	0.125	0.059	1.560	0.080	0.112	0.320	0.317	2.746	0.092	0.087	0.276	6.281
SMWvp62	2.11	0.026	1.840	8.467	1.889	0.031	0.538	0.457	0.210	42.022	0.624	0.056	ND	58.276
SMWp63	0.63	0.021	0.351	0.617	0.210	0.076	0.060	0.151	0.084	5.634	0.163	0.108	ND	8.109

Table S5: Factor loadings for the PCA analysis of OCPs found in South African wild bird eggs

EIG	*0.33*	*EIG*	*0.19*	*EIG*	*0.14*	*EIG*	*0.1*
NAME	**AX1**	**NAME**	**AX2**	**NAME**	**AX3**	**NAME**	**AX4**
ADk45	-1.0727	SMWvp62	-0.7615	SIs25	-0.5494	B/GHp3	-0.6361
ADk33	-0.9283	CPv56	-0.7214	SIs26	-0.5452	ADp36	-0.4835
WCr10	-0.8107	Svp66	-0.676	SIs15	-0.5297	ADk45	-0.475
WCr9	-0.7298	ADk43	-0.6451	ADk44	-0.5297	CPsb57	-0.4707
WCr7	-0.6953	Ssb65	-0.5742	SMWvp62	-0.5047	WCr9	-0.4646
WCr8	-0.667	Ssb64	-0.5638	SIs13	-0.4744	B/GHp2	-0.4333
ADp37	-0.6508	SMWp63	-0.5547	SMWp60	-0.4715	CPsb58	-0.3756
ADk44	-0.5937	SMWvp61	-0.4553	SIs23	-0.4283	BHHk4	-0.3481
ADp38	-0.5775	WCr8	-0.4518	SIs14	-0.4005	ADp35	-0.3473
ADp35	-0.5355	ADp39	-0.4277	SIs22	-0.3884	WCr7	-0.3433
ADk43	-0.4986	WCr10	-0.4273	SIs12	-0.3826	WCr10	-0.3356
ADk42	-0.4695	ADp38	-0.4206	SIs21	-0.3665	RCk49	-0.3295
ADk34	-0.4256	RCs46	-0.396	SIs16	-0.3196	SIs12	-0.3244
ADk40	-0.397	WCr7	-0.389	SIs19	-0.3065	RCk48	-0.2746
RCvp50	-0.3919	RCs47	-0.388	BCHs6	-0.2953	CPvp53	-0.2385
ADp36	-0.3663	RCvp50	-0.3549	SIs20	-0.286	WCr8	-0.2372
ADp39	-0.3592	ADp35	-0.3487	SIs11	-0.2835	ADk43	-0.2327
BCHs5	-0.353	RCk48	-0.3431	ADk43	-0.2757	SMWvp62	-0.224
BCHs6	-0.3263	WCr9	-0.3056	SMWp63	-0.2603	CPvp54	-0.2205
RCvp51	-0.2534	RCvp51	-0.3013	SIs18	-0.255	CPv56	-0.2178
SMWvp62	-0.2394	ADk40	-0.2955	CDsb52	-0.1941	ADp38	-0.2107
RCk48	-0.2269	CPsb57	-0.2925	SIs24	-0.1917	B/GHp1	-0.2025
SMWp60	-0.21	ADp37	-0.2911	BHHk4	-0.1787	SIs21	-0.1506
ADk41	-0.1655	CDsb52	-0.287	SIs17	-0.1572	SIs11	-0.1431
RCk49	-0.1539	SMWp60	-0.2843	WCr7	-0.1449	SIs26	-0.1303
BHHk4	-0.1519	CPsb58	-0.2307	CPsb57	-0.1444	ADp39	-0.1123
SIs15	-0.1059	RCk49	-0.2262	Ssb64	-0.1328	ADk40	-0.1035
Svp66	-0.0993	ADk44	-0.2078	CPv56	-0.1217	CEs27	-0.0773
SIs26	-0.0855	ADk45	-0.2054	SMWvp61	-0.1095	ADk33	-0.0709
SIs13	-0.0757	ADp36	-0.1937	Svp66	-0.1077	ADk34	-0.047
CEsb31	-0.0682	CEsb31	-0.1328	Ssb65	-0.0812	RCs47	-0.041
RCs46	-0.046	CPvp53	-0.113	BCHs5	-0.0718	BCHs5	-0.0394
CEp29	-0.0301	CPvp54	-0.0963	ADk45	-0.0582	CEs28	-0.0323
SIs24	-0.0282	ADk41	-0.0406	WCr8	-0.04	SIs18	-0.0152
SMWp63	-0.0277	CPvd55	-0.0063	RCk48	-0.0398	RCvp50	0.005
B/GHp1	-0.0246	SIs26	-0.0048	CPvp53	-0.0325	ADk44	0.0054
RCs47	-0.0207	CEp30	0.0505	RCk49	-0.0212	SIs14	0.0474
SIs22	-0.0198	CEsb32	0.0755	WCr10	-0.0137	SMWvp61	0.0572
SIs18	-0.0038	SIs25	0.1414	RCvp50	0.0025	ADp37	0.0884
Ssb64	0.005	ADk42	0.1774	WCr9	0.0203	RCs46	0.0889
CEp30	0.0374	BHHk4	0.1902	ADk40	0.0209	ADk42	0.0983
SIs23	0.0412	SIs14	0.2195	ADk33	0.095	BCHs6	0.1063
CEs27	0.0481	SIs23	0.2442	CPsb58	0.1108	SIs23	0.1076
SIs19	0.0633	SIs20	0.2493	RCs46	0.1109	SIs22	0.1456
SIs16	0.0823	CEp29	0.2497	ADk41	0.15	CPvd55	0.1483
SIs12	0.1473	CEs28	0.2909	CPvd55	0.1574	SIs16	0.1521
SIs17	0.1595	SIs24	0.3104	CEs27	0.1762	SIs17	0.1638
SIs14	0.1764	SIs19	0.3813	ADk42	0.1864	SIs24	0.1754
SIs25	0.207	SIs17	0.3817	ADp36	0.2239	SIs13	0.1967
CEsb32	0.2257	B/GHp3	0.4246	ADp38	0.2424	Svp66	0.2004
SIs11	0.2448	BCHs5	0.429	ADp39	0.2451	SIs20	0.2017
CEs28	0.2509	ADk34	0.4328	RCs47	0.2656	SIs19	0.2026
SIs21	0.2581	SIs11	0.4395	CPvp54	0.2684	SMWp60	0.2106
SIs20	0.2918	SIs12	0.4428	B/GHp2	0.3021	SIs15	0.2382
CDsb52	0.3453	SIs16	0.4958	RCvp51	0.3118	CEp29	0.2903
B/GHp2	0.3564	B/GHp1	0.4973	ADp35	0.3807	ADk41	0.319
Ssb65	0.5124	SIs18	0.5015	CEs28	0.4175	SMWp63	0.3818
SMWvp61	0.5452	SIs21	0.5063	ADp37	0.4698	CDsb52	0.4245
B/GHp3	0.6211	SIs22	0.5133	ADk34	0.4886	CEsb32	0.4585
CPvd55	0.9431	CEs27	0.5137	CEp29	0.634	SIs25	0.5242
CPvp53	0.9852	SIs15	0.7081	B/GHp3	0.6721	CEp30	0.5657
CPvp54	1.1975	SIs13	0.7801	CEsb32	0.7702	RCvp51	0.6275
CPv56	1.4949	BCHs6	0.84	CEp30	0.7865	Ssb64	0.6476
CPsb58	1.6491	B/GHp2	0.9206	CEsb31	0.8291	CEsb31	0.7396
CPsb57	1.9961	ADk33	1.007	B/GHp1	1.3555	Ssb65	0.7699

Table S6: Factor scores for the PCA analysis of OCPs found in South African wild bird eggs

EIG	***0.33***	***EIG***	***0.19***	***EIG***	***0.14***	***EIG***	***0.1***
NAME	**AX1**	**NAME**	**AX2**	**NAME**	**AX3**	**NAME**	**AX4**
p.p'-DDE	-1.5626	α-HCH	-1.2976	γ-HCH	-1.3494	Oxychlordane	-2.2898
β-HCH	-1.2172	γ-HCH	-1.1721	*p.p'*-DDD	-0.9973	β-HCH	-1.5962
α-HCH	-0.897	*Cis*-nonachlor	-1.0992	*Trans*-nonachlor	-0.649	*Cis*-nonachlor	-1.3254
γ-HCH	-0.6627	*Cis*-chlordane	-1.0725	*p.p'*-DDT	-0.586	*Cis*-chlordane	-0.7628
p.p'-DDD	-0.5127	*Trans*-chlordane	-0.9232	*Trans*-chlordane	-0.446	*Trans*-nonachlor	-0.5488
p.p'-DDT	-0.0581	HCB	-0.4758	*Cis*-chlordane	-0.4179	*p.p'*-DDE	-0.5395
Mirex	0.4532	β-HCH	-0.0395	Oxychlordane	-0.2601	γ-HCH	0.1932
Trans-chlordane	0.4984	Oxychlordane	0.0289	*p.p'*-DDE	0.283	Mirex	0.5279
Cis-chlordane	0.7486	Mirex	0.4088	*Cis*-nonachlor	0.3643	*p.p'*-DDD	0.7311
Oxychlordane	0.7989	*Trans*-nonachlor	0.5513	β-HCH	0.4309	*p.p'*-DDT	0.8113
HCB	0.87	*p.p'*-DDE	0.6844	α-HCH	0.4964	α-HCH	0.9368
Cis-nonachlor	0.907	*p.p'*-DDD	0.9801	HCB	1.0799	HCB	0.9585
Trans-nonachlor	1.5433	*p.p'*-DDT	1.9125	Mirex	2.264	*Trans*-chlordane	1.9345

Table S7: PCB Concentrations (ng g^{-1} wm) in wild bird eggs collected in South Africa

Species:	PCB-31	PCB-28	PCB-52	PCB-47	PCB-74	PCB-66	PCB-56	PCB-101	PCB-99	PCB-110	PCB-151	PCB-149	PCB-153	PCB-141
LOD	0.01	0.01	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.04	0.02	0.02	0.03	0.01
Recovery	91-117	78-115	78-97	89-113	81-11	76-99	71-99	92-108	83-99	77-110	77-97	88-104	85-100	69-82
SIs11	0.08	0.34	0.15	0.20	0.46	0.53	ND	0.17	0.89	0.19	0.09	0.44	10.04	0.13
SIs12	0.10	0.38	0.16	0.21	0.61	0.62	ND	0.20	1.04	0.14	0.09	0.51	11.86	0.14
SIs13	0.11	0.47	0.20	0.23	0.81	0.69	ND	0.31	1.18	0.27	0.19	0.68	11.37	0.18
SIs14	0.21	1.56	0.16	0.58	1.87	1.76	ND	0.25	1.53	0.16	0.22	0.73	13.55	0.23
SIs15	0.08	0.34	0.12	0.17	0.58	0.50	ND	0.20	0.84	0.20	0.10	0.44	7.93	0.12
SIs16	0.11	0.38	0.18	0.15	1.08	0.59	ND	0.32	1.00	0.45	0.26	0.70	10.56	0.18
SIs17	0.18	0.81	0.12	0.24	1.75	1.19	ND	0.21	0.88	0.14	0.13	0.48	7.97	0.09
SIs18	0.06	0.38	0.31	0.10	0.59	0.42	ND	0.09	0.57	0.07	0.03	0.13	5.58	0.03
SIs19	0.34	1.18	0.18	0.28	0.89	0.79	ND	0.13	0.58	0.17	0.06	0.34	5.62	0.08
SIs20	0.03	0.13	0.11	0.09	0.14	0.13	ND	0.07	0.24	0.13	0.09	0.28	3.58	0.05
SIs21	0.20	0.87	0.16	0.29	0.84	0.82	ND	0.16	0.85	0.14	0.14	0.66	12.47	0.21
SIs22	0.16	0.51	0.18	0.23	0.50	0.49	ND	0.28	0.97	0.36	0.26	0.90	14.49	0.22
SIs23	0.10	0.49	0.14	0.28	0.64	0.63	ND	0.25	0.88	0.05	0.26	0.72	7.98	0.20
SIs24	0.09	0.29	0.11	0.13	0.87	0.45	ND	0.19	1.72	0.13	0.09	0.43	14.42	0.08
SIs25	0.14	0.72	0.17	0.29	1.18	1.03	ND	0.77	3.18	1.24	0.23	1.10	14.87	0.27
SIs26	0.12	0.53	0.15	0.30	0.60	0.68	ND	0.24	0.79	0.26	0.17	0.59	7.44	0.16
CEs27	0.04	0.15	0.11	0.11	0.21	0.21	ND	0.04	0.36	0.10	0.13	0.35	12.87	0.04
CEs28	0.03	0.09	0.08	0.12	0.17	0.18	ND	0.04	0.28	0.06	0.02	0.04	6.25	ND
CEp29	0.03	0.02	0.08	0.04	0.06	0.04	ND	0.04	0.06	ND	ND	ND	1.37	ND
CEp30	ND	ND	0.07	0.05	0.02	0.03	ND	0.02	0.02	ND	ND	ND	0.44	ND
CEsb31	ND	0.02	0.09	0.03	ND	0.12	ND	0.03	0.02	ND	ND	ND	0.34	ND
CEsb32	ND	0.05	0.07	0.04	0.02	0.02	ND	0.03	0.03	ND	ND	ND	0.90	ND
ADk33	0.19	1.61	0.25	0.33	4.36	1.72	ND	0.45	3.68	0.73	0.50	0.65	54.27	0.20
ADk34	0.14	1.19	0.27	0.46	4.81	2.05	ND	0.69	6.92	0.86	0.63	0.83	136.07	0.27
ADp35	0.44	2.41	0.15	0.23	5.32	1.84	ND	0.34	4.21	0.14	0.11	0.33	51.71	0.08
ADp36	0.68	7.23	0.23	0.56	7.70	4.81	ND	0.44	4.07	0.26	0.43	0.68	65.91	0.26
ADp37	0.11	0.76	0.14	0.23	1.27	0.89	ND	0.29	2.38	0.25	0.16	0.26	17.30	0.12
ADp38	1.18	4.99	0.23	0.15	6.97	2.50	0.05	0.53	3.91	0.21	0.19	0.41	73.73	0.19
ADp39	0.90	4.50	0.14	0.04	5.92	3.17	0.24	0.43	3.95	0.22	0.30	0.59	69.40	0.22
ADk40	2.33	8.30	0.50	0.12	10.47	6.76	0.07	1.04	9.84	0.92	0.56	0.91	192.84	0.41
ADk41	ND	0.31	0.15	0.21	0.75	0.51	0.04	0.32	1.96	0.18	0.08	0.12	18.40	0.09
ADk42	0.04	0.24	0.15	0.04	1.89	0.36	0.02	0.33	1.65	0.36	0.10	0.31	50.84	0.09
ADk43	3.82	34.53	1.15	0.39	20.15	18.30	0.08	2.80	8.78	5.11	3.58	5.09	276.42	1.63
ADk44	0.47	1.15	0.31	0.05	4.12	1.33	0.02	1.19	4.44	2.51	1.29	2.69	147.04	0.81
ADk45	0.32	7.40	0.46	ND	9.16	6.09	0.03	0.63	5.50	0.85	0.64	0.50	76.47	0.23
WCr7	0.57	4.12	0.48	0.08	7.75	5.56	0.02	0.65	6.67	0.22	0.03	0.33	57.66	0.10
WCr8	1.74	7.89	0.93	0.06	13.29	10.64	0.03	0.97	12.04	0.37	0.07	0.60	93.26	0.19
WCr9	1.13	5.76	0.68	0.08	9.64	7.62	0.05	0.82	8.56	0.29	0.10	0.43	67.37	0.15
WCr10	1.05	8.60	0.44	0.12	17.16	10.03	0.04	0.87	13.04	0.30	0.05	0.51	124.37	0.14
BCHs5	0.06	0.17	0.03	0.03	0.29	0.18	ND	0.07	0.39	0.08	0.13	0.31	10.81	0.05
BCHs6	0.09	0.09	0.07	0.04	0.16	0.11	ND	0.09	0.28	0.08	0.14	0.36	11.89	0.06
BHHk4	ND	0.09	0.08	0.06	0.19	0.09	ND	0.08	0.18	ND	ND	0.03	5.17	0.01
B/GHp1	0.04	0.46	0.09	0.03	0.54	0.50	ND	0.09	1.08	ND	ND	0.06	16.45	0.02
B/GHp2	0.41	0.51	0.11	ND	1.22	0.66	ND	0.09	1.43	ND	ND	0.09	51.53	0.04
B/GHp3	0.47	1.85	0.08	0.03	2.53	1.69	ND	0.08	1.50	ND	ND	0.05	45.27	0.03
CPvp53	ND	1.46	0.50	0.82	1.15	2.05	ND	1.70	2.09	3.27	0.44	3.12	24.15	0.31
CPvp54	ND	0.27	0.15	0.23	0.25	0.43	ND	0.46	0.65	0.62	0.23	2.50	16.07	0.04
CPvd55	ND	0.07	0.13	0.08	0.11	0.10	ND	0.17	0.15	0.18	0.03	0.26	1.91	0.02
CPv56	ND	0.10	0.12	0.10	0.11	0.66	ND	0.24	0.39	0.16	0.05	0.32	2.39	0.03
CPsb57	ND	0.12	0.12	0.13	ND	1.94	ND	0.23	1.09	ND	ND	0.48	5.14	0.05
CPsb58	ND	0.08	0.13	0.12	0.15	0.94	ND	0.13	1.10	0.30	ND	0.39	5.37	0.03
RCs46	0.05	0.44	0.14	0.10	0.24	0.33	ND	0.10	0.16	0.10	0.06	0.14	1.33	0.02
RCs47	0.09	0.41	0.12	0.11	0.30	0.29	ND	0.22	0.15	0.05	0.05	0.12	1.22	0.03
RCk48	0.63	1.55	0.46	0.43	0.73	1.31	ND	0.44	0.65	0.40	0.26	0.47	3.21	ND
RCk49	0.42	1.73	0.45	0.51	0.79	1.42	ND	0.45	0.70	0.22	0.28	0.52	0.49	0.07
RCvp50	0.82	3.95	0.65	1.02	1.62	2.56	ND	0.35	0.73	0.26	0.15	0.34	2.93	0.05
RCvp51	0.10	0.43	0.08	0.11	0.22	0.36	ND	0.06	0.13	0.07	0.04	0.06	0.60	0.02
CDsb52	ND	ND	0.04	0.17	ND	ND	ND	0.02	ND	ND	ND	ND	0.12	0.01
Ssb64	0.04	0.07	0.11	0.08	0.07	0.06	ND	0.11	0.08	0.06	ND	ND	1.27	0.02
Ssb65	0.04	0.03	0.09	0.12	0.05	0.04	ND	0.08	0.06	ND	ND	0.10	11.69	0.01
Svp66	ND	ND	0.15	0.08	0.12	0.11	ND	0.52	0.40	0.12	0.08	0.47	7.93	0.12
SMWp60	0.06	0.23	0.12	0.04	0.56	0.53	ND	0.66	0.75	0.22	0.02	0.43	5.70	0.09
SMWvp61	0.03	0.09	0.15	0.15	0.34	0.29	ND	0.22	0.30	ND	ND	0.11	0.94	0.02
SMWvp62	0.38	4.75	0.31	2.76	7.06	6.52	ND	2.95	4.63	0.88	ND	1.18	13.04	0.33
SMWp63	0.03	0.15	0.14	0.14	0.31	0.24	ND	0.23	0.31	0.06	0.02	0.07	2.00	0.03

Table S7 continued: PCB Concentrations (ng g^{-1} ww) in wild bird eggs collected in South Africa

Species:	PCB-137	PCB-138	PCB-187	PCB-183	PCB-128	PCB-180	PCB-170	PCB-199	PCB-196	PCB-194	PCB-206	PCB-209	PCB-105	PCB-114
LOD	0.02	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.18	0.01	0.01
Recovery	66-83	68-85	71-102	58-72	72-87	53-84	64-90	83-111	61-93	88-124	90-115	90-117	100.18	99.04
SIs11	0.17	6.45	3.34	1.80	0.57	10.36	4.00	0.16	3.01	3.97	4.80	0.64	0.92	0.07
SIs12	0.20	7.68	5.23	2.11	2.11	11.12	4.60	0.16	3.75	4.47	6.26	0.80	1.14	0.08
SIs13	0.19	7.25	2.72	1.79	0.82	10.60	4.08	0.11	3.47	3.13	4.14	0.57	1.52	0.14
SIs14	0.25	8.98	4.04	2.74	0.94	13.19	5.37	0.16	3.31	4.13	4.18	0.64	1.98	0.16
SIs15	0.14	4.85	1.72	1.20	0.55	7.04	2.76	0.08	2.29	2.14	2.97	0.37	1.04	0.09
SIs16	0.15	6.92	4.08	1.70	0.67	9.72	3.90	0.14	3.56	3.87	7.41	1.16	1.28	0.11
SIs17	0.12	4.94	2.77	1.28	0.50	7.53	2.97	0.09	2.47	2.26	3.84	0.57	1.12	0.09
SIs18	0.09	3.13	0.91	0.74	0.37	4.52	1.87	0.05	1.73	1.41	2.92	0.43	0.66	0.06
SIs19	0.12	3.65	1.47	0.82	0.47	4.30	1.86	0.05	1.41	1.29	2.09	0.28	0.73	0.05
SIs20	0.03	1.94	1.69	0.61	0.17	3.46	1.22	0.05	1.42	1.28	2.30	0.27	0.19	0.01
SIs21	0.15	8.11	3.41	2.16	0.76	12.74	4.87	0.13	5.58	4.43	9.08	1.00	1.08	0.08
SIs22	0.21	8.38	4.28	2.54	0.80	17.33	6.12	0.14	4.03	6.58	4.52	0.65	1.12	0.08
SIs23	0.16	5.35	3.11	1.11	0.56	6.59	2.51	0.11	3.29	2.90	6.52	1.09	0.83	0.06
SIs24	0.36	10.41	2.42	1.72	1.35	9.44	4.08	0.07	2.28	2.21	2.60	0.23	2.49	0.22
SIs25	0.39	10.31	4.37	2.42	1.23	12.91	5.05	0.12	2.94	3.79	3.65	0.54	2.98	0.22
SIs26	0.15	4.54	3.11	1.13	0.43	7.58	2.49	0.12	5.24	4.63	10.46	1.49	0.70	0.06
CEs27	0.05	5.72	2.43	1.23	0.41	9.01	3.18	0.09	5.73	3.33	4.88	0.46	0.34	0.02
CEs28	0.05	2.36	1.85	0.71	0.16	5.49	1.52	0.07	3.00	2.49	4.09	0.38	0.30	0.02
CEp29	ND	0.41	0.19	0.10	0.00	0.79	0.24	0.01	0.25	0.30	0.36	ND	0.06	ND
CEp30	ND	0.12	0.05	0.03	0.01	0.26	0.07	0.01	0.05	0.10	0.11	ND	0.02	ND
CEsb31	ND	0.09	0.04	0.02	0.01	0.21	0.04	ND	0.05	0.07	0.07	ND	0.01	ND
CEsb32	ND	0.19	0.07	0.07	ND	0.87	0.22	0.02	0.17	0.32	0.25	ND	0.02	ND
ADk33	0.09	29.35	8.95	6.00	3.11	32.72	12.61	0.38	8.24	5.69	5.26	0.45	5.97	0.56
ADk34	1.00	72.06	21.43	15.65	7.91	99.10	44.25	0.70	16.16	15.33	6.11	0.59	9.03	0.83
ADp35	0.39	18.62	4.37	3.13	2.57	26.39	10.83	0.25	7.03	6.01	7.42	1.02	6.29	0.62
ADp36	0.45	27.22	7.80	5.81	2.80	45.74	18.04	0.30	9.77	8.25	5.83	0.41	5.80	0.59
ADp37	0.27	7.70	1.71	1.12	1.07	8.95	3.70	0.14	2.69	2.19	2.47	0.21	2.52	0.23
ADp38	0.39	23.23	7.45	5.23	2.38	32.64	13.79	0.50	4.69	11.89	9.05	0.98	5.77	0.63
ADp39	0.37	22.71	6.26	4.68	2.25	29.46	12.43	0.36	3.78	8.81	5.75	0.52	4.65	0.57
ADk40	1.09	90.69	24.06	12.00	7.00	87.61	38.86	0.77	8.21	11.05	8.77	0.62	11.11	1.19
ADk41	0.11	6.35	2.04	1.51	0.62	8.78	3.62	0.11	1.12	2.26	1.29	ND	0.89	0.07
ADk42	0.18	12.14	8.74	4.17	1.37	23.77	9.56	0.60	4.67	7.21	8.00	0.47	2.58	0.23
ADk43	0.99	120.68	38.98	11.67	5.65	117.75	49.13	0.84	7.21	13.75	11.17	1.56	12.72	1.22
ADk44	0.52	52.69	15.58	8.20	3.80	72.40	28.20	0.87	8.43	10.28	8.87	0.89	6.34	0.93
ADk45	0.38	31.48	8.92	3.95	2.08	33.40	13.58	0.35	2.88	7.17	4.55	0.50	6.15	0.67
WCr7	0.48	30.80	5.54	2.56	1.52	20.86	9.31	0.18	2.51	5.42	4.89	0.66	5.78	0.55
WCr8	0.87	55.82	15.28	5.16	2.56	39.38	16.30	0.33	4.42	8.70	7.22	0.75	10.22	0.90
WCr9	0.63	39.40	10.81	3.55	1.87	27.29	11.16	0.25	3.09	6.56	5.55	0.63	7.75	0.67
WCr10	0.76	65.31	12.56	5.88	3.12	45.65	21.55	0.42	6.72	5.46	13.13	1.36	12.08	1.10
BCHs5	0.04	2.77	2.89	0.72	0.16	6.65	1.55	0.16	4.02	9.75	21.48	1.46	0.20	0.02
BCHs6	0.04	2.88	3.17	0.79	0.18	7.29	1.71	0.25	4.26	11.19	20.07	1.90	0.24	0.02
BHHk4	ND	0.16	0.71	0.41	0.04	2.61	0.82	0.02	0.69	2.45	3.54	0.49	0.20	0.03
B/GHp1	0.14	5.99	0.37	1.57	0.39	7.32	3.14	0.02	1.67	5.24	5.15	1.01	1.00	0.09
B/GHp2	0.13	9.74	2.01	2.75	0.42	31.57	7.24	0.07	4.21	11.66	15.18	2.83	1.88	0.18
B/GHp3	0.15	7.80	1.88	2.54	0.44	21.38	6.02	0.04	3.21	5.35	4.58	0.92	1.82	0.13
CPvp53	0.57	14.88	10.53	3.40	1.27	14.58	4.90	0.29	6.28	3.84	9.02	1.66	2.08	0.17
CPvp54	0.04	8.18	7.09	1.47	0.49	6.00	2.37	0.11	2.34	3.11	3.39	0.49	0.51	0.06
CPvd55	0.05	1.02	0.75	0.30	0.10	1.08	0.38	0.02	0.19	0.47	0.14	ND	0.12	0.02
CPv56	0.17	1.63	1.27	0.42	0.12	1.69	0.70	0.06	0.54	1.14	0.82	0.63	0.21	0.02
CPsb57	0.29	2.88	3.83	0.75	0.26	2.87	0.87	0.13	2.83	2.80	3.90	0.63	0.39	0.08
CPsb58	0.18	3.01	2.35	0.69	0.26	3.03	1.08	0.07	1.07	1.77	1.23	0.21	0.45	0.05
RCs46	0.02	0.97	0.39	0.18	0.08	0.62	0.27	0.02	0.21	0.45	0.19	ND	0.28	ND
RCs47	0.02	0.91	0.36	0.17	0.08	0.57	0.25	0.02	0.22	0.43	0.22	ND	0.26	ND
RCk48	0.05	3.04	0.65	0.25	0.31	1.03	0.49	0.01	0.16	0.35	0.08	ND	0.74	0.07
RCk49	0.07	3.29	0.69	0.27	0.34	1.09	0.52	0.01	0.16	0.37	0.07	ND	0.81	0.07
RCvp50	0.07	2.65	0.96	0.31	0.23	1.19	0.47	0.03	0.52	0.71	0.57	ND	0.80	0.09
RCvp51	0.02	0.55	0.15	0.07	0.06	0.27	0.11	0.02	0.10	0.22	0.09	ND	0.21	0.02
CDsb52	ND	0.06	0.02	ND	ND	0.06	ND	ND	0.02	0.04	0.02	ND	ND	ND
Ssb64	0.02	0.57	0.36	0.17	0.05	0.76	0.33	0.04	0.22	0.42	0.29	ND	0.05	ND
Ssb65	0.04	0.26	0.14	0.08	0.03	0.33	0.13	0.03	0.08	0.16	0.09	0.52	0.03	ND
Svp66	0.09	4.85	1.28	0.64	0.42	2.32	1.33	0.04	0.30	0.60	0.19	ND	0.25	ND
SMWp60	0.09	2.86	1.13	0.53	0.28	2.04	1.01	0.03	0.47	0.95	0.33	ND	0.54	ND
SMWvp61	0.04	0.70	0.19	0.10	0.10	0.40	0.17	ND	0.25	0.26	0.25	ND	0.25	ND
SMWvp62	0.65	1.17	1.49	0.89	1.89	4.79	2.66	0.06	0.81	1.99	1.09	0.16	5.83	0.45
SMWp63	0.04	0.99	0.38	0.18	0.11	0.83	0.35	0.02	0.19	0.39	0.18	ND	0.17	ND

Table S7 continued: PCB Concentrations (ng g^{-1} wm) in wild bird eggs collected in South Africa

Species:	PCB-118	PCB-123	PCB-156	PCB-157	PCB-167	PCB-189
LOD	0.01	0.01	0.01	0.01	0.00	0.00
Recovery	102.45		99.30	100.84	98.27	106.43
SIs11	2.51	0.09	1.14	0.21	0.45	0.17
SIs12	3.04	0.12	1.42	0.26	0.47	0.19
SIs13	3.90	0.16	1.48	0.29	0.36	0.17
SIs14	4.63	0.21	1.68	0.33	0.56	0.21
SIs15	2.59	0.11	0.96	0.19	0.32	0.11
SIs16	3.07	0.15	1.23	0.23	0.50	0.16
SIs17	2.68	0.13	0.95	0.17	0.32	0.11
SIs18	1.85	0.08	0.72	0.16	0.28	0.08
SIs19	1.86	0.07	0.66	0.14	0.24	0.08
SIs20	0.57	0.02	0.29	0.05	0.12	0.05
SIs21	2.87	0.12	1.43	0.25	0.54	0.19
SIs22	3.13	0.12	1.46	0.25	0.61	0.25
SIs23	2.24	0.10	0.89	0.19	0.35	0.11
SIs24	7.16	0.28	2.35	0.53	0.75	0.16
SIs25	6.66	0.29	1.89	0.39	0.67	0.18
SIs26	2.07	0.09	0.77	0.15	0.29	0.13
CEs27	1.66	0.05	1.09	0.18	0.55	0.21
CEs28	1.04	0.05	0.54	0.11	0.31	0.12
CEp29	0.30	0.01	0.10	0.02	0.08	0.02
CEp30	0.13	ND	0.03	0.01	0.03	0.01
CEsb31	0.07	ND	0.02	0.01	0.03	0.01
CEsb32	0.10	ND	0.06	0.01	0.06	0.02
ADk33	19.30	0.76	4.47	1.20	2.50	0.37
ADk34	32.06	1.30	11.28	2.70	6.40	1.13
ADp35	25.70	0.68	6.53	1.54	3.17	0.47
ADp36	19.28	0.72	6.79	1.46	3.37	0.73
ADp37	9.17	0.28	2.45	0.57	1.17	0.20
ADp38	21.36	0.40	7.01	1.91	3.69	0.67
ADp39	17.09	0.29	5.55	1.52	2.97	0.52
ADk40	38.44	0.81	10.89	2.86	6.19	0.87
ADk41	3.61	0.06	1.18	0.31	0.96	0.10
ADk42	8.73	0.22	3.01	0.81	2.00	0.24
ADk43	40.10	0.81	10.43	2.96	5.63	0.85
ADk44	26.31	0.48	7.25	2.31	4.49	0.60
ADk45	19.34	0.44	3.55	1.40	2.58	0.27
WCr7	14.80	0.44	2.66	0.80	1.74	0.19
WCr8	25.95	0.71	4.21	1.32	2.53	0.27
WCr9	19.08	0.53	3.04	0.97	2.18	0.19
WCr10	31.43	0.85	5.61	1.82	3.24	0.40
BCHs5	0.82	0.02	0.72	0.17	0.60	0.15
BCHs6	0.95	0.02	0.89	0.20	0.71	0.19
BHHk4	0.96	0.02	0.47	0.12	0.31	0.06
B/GHp1	4.72	0.08	1.84	0.30	1.07	0.26
B/GHp2	5.34	0.09	3.58	0.69	1.55	0.49
B/GHp3	4.65	0.06	3.19	0.60	1.40	0.52
CPvp53	6.57	0.20	2.15	0.67	1.33	0.38
CPvp54	2.63	0.04	0.81	0.19	0.52	0.13
CPvd55	1.88	ND	0.15	0.05	0.14	0.03
CPv56	2.03	0.03	0.22	0.06	0.21	0.05
CPsb57	2.02	0.11	0.40	0.19	0.35	0.07
CPsb58	1.96	0.08	0.39	0.13	0.39	0.08
RCs46	0.72	0.04	0.18	0.04	0.12	0.02
RCs47	0.65	0.04	0.15	0.04	0.10	0.02
RCk48	1.92	0.10	0.29	0.08	0.20	0.02
RCk49	2.07	0.09	0.32	0.09	0.19	0.03
RCvp50	1.94	0.12	0.39	0.09	0.30	0.03
RCvp51	0.53	0.03	0.10	0.03	0.08	0.01
CDsb52	0.07	ND	ND	ND	0.02	ND
Ssb64	0.29	ND	0.10	0.02	0.09	0.02
Ssb65	1.86	ND	0.04	0.01	0.05	0.00
Svp66	1.61	0.06	0.63	0.11	0.39	0.07
SMWp60	1.30	0.08	0.50	0.09	0.36	0.06
SMWvp61	0.37	ND	0.12	0.04	0.11	0.01
SMWvp62	NA	0.49	3.05	0.84	1.03	0.18
SMWp63	0.63	ND	0.20	0.05	0.15	0.03

Table S8: Factor scores for the PCA analysis of PCBs in wild bird eggs from South Africa

EIG	*0.3174*		*0.2188*		*0.1069*		*0.071*
NAME	**AX1**	**NAME**	**AX2**	**NAME**	**AX3**	**NAME**	**AX4**
CB170	-1.5768	CB194	-1.5543	CB31	-1.7408	CB118	-3.0252
CB180	-1.5111	CB196	-1.5295	CB209	-1.4908	CB138	-1.7522
CB156	-1.442	CB209	-1.5239	CB56	-1.4693	CB151	-1.5854
CB183	-1.3967	CB206	-1.4854	CB194	-0.8971	CB153	-0.9507
CB157	-1.3598	CB199	-1.4833	CB206	-0.8065	CB56	-0.9456
CB153	-1.1552	CB189	-1.2124	CB52	-0.799	CB199	-0.8791
CB138	-1.0112	CB187	-0.8848	CB28	-0.7369	CB167	-0.5556
CB105	-0.911	CB183	-0.7698	CB167	-0.7185	CB110	-0.4338
CB189	-0.8811	CB180	-0.7347	CB189	-0.5448	CB52	-0.3638
CB196	-0.8662	CB56	-0.507	CB153	-0.47	CB31	-0.3388
CB206	-0.8646	CB151	-0.3898	CB74	-0.4159	CB187	-0.2255
CB114	-0.7832	CB153	-0.3755	CB66	-0.2612	CB180	-0.1847
CB194	-0.7724	CB141	-0.3746	CB196	-0.1437	CB170	-0.1484
CB167	-0.7559	CB149	-0.3245	CB180	-0.1331	CB183	-0.0765
CB187	-0.6697	CB167	-0.2826	CB118	-0.1281	CB74	-0.0344
CB99	-0.6628	CB47	-0.245	CB114	-0.0513	CB101	0.1486
CB128	-0.6498	CB170	-0.2409	CB47	0.0116	CB141	0.3591
CB118	-0.4458	CB118	-0.0877	CB199	0.0595	CB209	0.3763
CB74	-0.4219	CB52	-0.0364	CB157	0.0909	CB128	0.4035
CB28	0.0186	CB110	-0.0158	CB156	0.1152	CB149	0.4515
CB199	0.0238	CB156	0.0818	CB123	0.411	CB28	0.477
CB123	0.025	CB137	0.2203	CB170	0.4412	CB114	0.511
CB31	0.3526	CB101	0.3284	CB137	0.4864	CB157	0.546
CB66	0.3877	CB157	0.3666	CB105	0.6042	CB123	0.5803
CB137	0.413	CB138	0.4136	CB99	0.6174	CB105	0.7122
CB209	0.4522	CB128	0.7514	CB101	0.64	CB194	0.717
CB141	0.8669	CB114	0.9168	CB183	0.7196	CB156	0.7564
CB149	0.8933	CB123	1.0098	CB151	0.935	CB99	0.7967
CB151	0.9796	CB31	1.2959	CB138	0.9998	CB137	0.7991
CB56	1.1983	CB99	1.2965	CB141	1.0863	CB189	0.8216
CB110	1.21	CB66	1.5481	CB187	1.515	CB66	0.8947
CB101	1.4215	CB105	1.569	CB110	1.5187	CB196	1.0009
CB47	1.5573	CB74	1.7174	CB128	1.8305	CB47	1.0694
CB52	1.5575	CB28	1.7729	CB149	1.9605	CB206	1.1228

Table S9: Factor loadings for the PCA analysis of PCBs in wild bird eggs from South Africa

EIG	*0.3174*		*0.2188*		*0.1069*		*0.071*
NAME	**AX1**	**NAME**	**AX2**	**NAME**	**AX3**	**NAME**	**AX4**
B/GHp2	-1.1446	BCHs6	-1.0149	B/GHp2	-0.7234	Ssb65	-0.4487
B/GHp3	-0.9947	BCHs5	-0.8576	B/GHp3	-0.7004	ADk45	-0.4168
B/GHp1	-0.7729	CEs27	-0.6546	CEp29	-0.6331	ADk44	-0.4103
ADk34	-0.7613	CPsb57	-0.6544	CEsb32	-0.5396	Svp66	-0.364
WCr10	-0.7354	BHHk4	-0.5854	B/GHp1	-0.4863	ADp39	-0.3381
ADp35	-0.7209	CPvp54	-0.5628	BHHk4	-0.473	ADk43	-0.3176
ADk42	-0.7012	CEs28	-0.5573	CEsb31	-0.4197	ADk40	-0.3092
ADk40	-0.648	CEsb32	-0.5399	CDsb52	-0.3575	CPvd55	-0.3085
ADk45	-0.6184	SIs20	-0.5096	CEp30	-0.3534	RCk48	-0.2503
WCr8	-0.5994	Svp66	-0.4697	Ssb65	-0.3516	ADk42	-0.2102
ADp38	-0.5923	CEp30	-0.4454	Ssb64	-0.3358	CEp29	-0.1699
ADp39	-0.5715	CEp29	-0.4164	CEs28	-0.3052	WCr9	-0.1664
ADk44	-0.5574	CPv56	-0.3922	ADp38	-0.2825	CEp30	-0.1624
WCr7	-0.5329	CPvp53	-0.385	ADp39	-0.2438	CDsb52	-0.1596
ADp36	-0.5286	SIs26	-0.3807	RCvp51	-0.2389	ADk33	-0.1344
ADk33	-0.4777	ADk42	-0.3347	WCr10	-0.2351	ADk41	-0.1301
WCr9	-0.4477	Ssb65	-0.3319	WCr7	-0.2243	RCk49	-0.1256
BCHs5	-0.402	CDsb52	-0.3211	WCr9	-0.2197	ADp38	-0.1237
SIs24	-0.3961	SIs11	-0.3192	BCHs5	-0.2143	WCr8	-0.1234
CEs28	-0.3166	SIs22	-0.2975	BCHs6	-0.2131	RCs46	-0.1142
BCHs6	-0.3143	CPsb58	-0.2879	SIs18	-0.2004	ADk34	-0.1003
CEs27	-0.2859	SIs12	-0.277	WCr8	-0.191	Ssb64	-0.0976
ADp37	-0.2345	SIs23	-0.2739	SMWvp61	-0.1503	SMWp60	-0.0858
SIs12	-0.2275	SIs16	-0.2591	RCvp50	-0.1479	RCvp51	-0.0774
BHHk4	-0.186	CEsb31	-0.245	RCs47	-0.1342	SMWp63	-0.0656
ADk43	-0.1519	Ssb64	-0.2428	ADp35	-0.1107	RCs47	-0.0624
SIs21	-0.1517	SIs21	-0.2262	SIs19	-0.0974	CEsb31	-0.0574
SIs11	-0.1269	CPvd55	-0.1883	SMWp63	-0.0477	ADp37	-0.0379
ADk41	-0.1013	B/GHp2	-0.1821	RCs46	-0.0415	CPvp54	-0.0293
SIs18	-0.0983	ADk41	-0.1082	SIs21	-0.0056	CEsb32	-0.0225
SIs22	-0.0847	SIs13	-0.1042	SIs17	-0.0054	CPv56	-0.0225
SIs14	-0.0815	B/GHp1	-0.1013	SIs26	0.0156	WCr7	-0.0201
SIs13	-0.0605	SIs15	-0.0954	ADk40	0.0359	WCr10	-0.0179
CPsb57	-0.0559	SIs18	-0.0462	RCk48	0.0532	SIs24	-0.0097
SIs16	-0.0535	ADk44	-0.021	CEs27	0.0549	CEs27	0.0139
SIs15	-0.0398	ADk34	0.0015	SIs23	0.0576	ADp35	0.0292
SIs17	-0.0311	SIs25	0.0817	ADp36	0.0884	SIs20	0.0414
SIs25	-0.0064	SIs24	0.0909	SIs20	0.1073	ADp36	0.0533
SIs23	0.0282	SIs17	0.0915	SIs14	0.1125	SIs22	0.0534
CPsb58	0.0459	SIs14	0.1048	SIs11	0.1215	SIs25	0.0534
SIs26	0.0901	SMWp63	0.1217	ADk45	0.1272	BCHs6	0.0558
CPvp54	0.1041	ADk33	0.1742	CPsb57	0.1381	SIs16	0.0583
SIs19	0.1731	SMWp60	0.2157	SIs12	0.1672	RCvp50	0.0811
CPvp53	0.1863	ADp37	0.2216	SIs15	0.1754	SIs13	0.0813
SIs20	0.2058	SMWvp61	0.2363	ADp37	0.1947	SIs15	0.0912
SMWp60	0.2804	SIs19	0.2541	SIs16	0.1959	B/GHp2	0.1048
Svp66	0.3449	B/GHp3	0.2585	RCk49	0.1986	BCHs5	0.1086
CEp29	0.3749	RCs46	0.2846	ADk42	0.2112	B/GHp1	0.1222
CPv56	0.3983	ADp35	0.3097	ADk43	0.217	SIs11	0.1251
CEsb32	0.4951	ADp38	0.316	SIs13	0.2196	SIs18	0.1281
SMWvp62	0.5204	RCs47	0.3242	CPv56	0.2259	SIs19	0.1439
CPvd55	0.536	ADp39	0.3645	SIs22	0.2354	SIs17	0.1485
Ssb64	0.542	ADp36	0.4467	SMWvp62	0.241	B/GHp3	0.1494
SMWp63	0.6142	ADk40	0.5043	SIs24	0.263	SMWvp61	0.1552
RCs46	0.6739	ADk45	0.5588	ADk41	0.2907	CPsb58	0.1606
SMWvp61	0.6911	ADk43	0.5682	SMWp60	0.2935	CEs28	0.1632
RCs47	0.7014	RCvp51	0.5711	CPvd55	0.3299	SIs12	0.1763
RCvp51	0.8142	WCr10	0.6278	CPsb58	0.3566	SIs14	0.1818
RCvp50	0.8152	WCr7	0.6336	ADk33	0.3578	SIs23	0.182
CEp30	0.8378	WCr9	0.6686	SIs25	0.4519	CPvp53	0.1986
Ssb65	0.8654	WCr8	0.728	ADk44	0.4828	SIs21	0.2038
RCk48	0.8827	RCvp50	0.8931	ADk34	0.5442	BHHk4	0.21
RCk49	0.9882	RCk49	0.9934	CPvp54	0.6216	SIs26	0.2399
CEsb31	1.0074	RCk48	1.0075	Svp66	0.7319	CPsb57	0.3986
CDsb52	1.5944	SMWvp62	1.0364	CPvp53	0.7651	SMWvp62	1.5769

Table S10: Factor loadings for the PCA analysis of organohalogens in wild bird eggs from South Africa

EIG	*0.3371*	*EIG*	*0.1715*	*EIG*	*0.1148*	*EIG*	*0.0675*
NAME	**AX1**	**NAME**	**AX2**	**NAME**	**AX3**	**NAME**	**AX4**
CDsb52	-1.0484	CDsb52	-0.7085	SMWvp62	-0.6095	SMWvp62	-0.58
CEsb31	-0.7228	CPv56	-0.6646	RCvp50	-0.5958	CPsb57	-0.4748
RCs47	-0.7189	SMWvp61	-0.6015	RCk49	-0.559	SMWvp61	-0.4045
CEp30	-0.69	CPsb58	-0.5875	RCk48	-0.5054	CPv56	-0.3942
Ssb65	-0.6837	RCk48	-0.562	RCvp51	-0.4748	CPvp53	-0.3902
SIs18	-0.6814	RCs46	-0.5511	RCs47	-0.4397	CPsb58	-0.3888
Ssb64	-0.6191	CEsb31	-0.5473	SMWp60	-0.2669	RCk49	-0.2666
CPvd55	-0.5328	CPsb57	-0.5164	SMWp63	-0.2617	SIs25	-0.2447
SIs19	-0.5204	RCk49	-0.5127	Svp66	-0.2501	ADk43	-0.2416
SIs15	-0.4746	Ssb65	-0.508	RCs46	-0.2379	CPvp54	-0.2354
SIs11	-0.459	RCvp51	-0.489	WCr9	-0.2343	RCk48	-0.2246
SIs13	-0.4477	Svp66	-0.4717	SIs24	-0.2179	SIs14	-0.2164
SMWvp61	-0.4394	ADk41	-0.4663	WCr10	-0.2108	Svp66	-0.1721
SIs21	-0.4303	CEp30	-0.4459	SIs17	-0.1856	SIs26	-0.1707
BHHk4	-0.4237	CPvp54	-0.3811	WCr8	-0.183	SIs21	-0.1703
SIs17	-0.398	RCvp50	-0.3667	WCr7	-0.1783	RCvp50	-0.1666
SIs16	-0.392	ADp39	-0.3524	ADk40	-0.1725	ADk40	-0.1586
SIs26	-0.3906	RCs47	-0.3176	Ssb64	-0.1716	ADk44	-0.1535
SMWp63	-0.3824	SMWp60	-0.302	SIs25	-0.1677	SIs23	-0.1374
SIs23	-0.3788	Ssb64	-0.3012	SIs26	-0.1634	CPvd55	-0.1372
SIs25	-0.3748	ADk43	-0.2978	SIs14	-0.1621	SIs11	-0.1312
SIs22	-0.3688	SMWp63	-0.2757	SIs19	-0.1553	SMWp60	-0.1218
SIs14	-0.3663	CPvp53	-0.2626	SMWvp61	-0.1497	WCr7	-0.1172
Svp66	-0.3433	CPvd55	-0.2551	ADk45	-0.1353	SIs17	-0.1067
SIs20	-0.3397	SMWvp62	-0.2502	SIs18	-0.1321	SIs12	-0.1031
RCvp51	-0.3374	WCr8	-0.1755	ADk44	-0.1176	SIs19	-0.1018
CPv56	-0.3342	ADp37	-0.1031	ADp37	-0.1156	SIs22	-0.0995
CEsb32	-0.3253	WCr10	-0.0868	SIs16	-0.1142	SIs13	-0.0944
SIs24	-0.3161	WCr9	-0.0753	SIs22	-0.103	ADp36	-0.0932
SMWp60	-0.2793	CEsb32	-0.0744	SIs15	-0.0966	WCr8	-0.0914
RCs46	-0.2625	ADk45	-0.0661	SIs13	-0.0914	SMWp63	-0.089
CPsb57	-0.2559	ADp36	-0.0544	CEsb31	-0.0857	SIs15	-0.0841
CPsb58	-0.2307	ADk44	-0.0331	SIs23	-0.0766	SIs24	-0.08
CEp29	-0.1883	ADp38	-0.0068	Ssb65	-0.0669	SIs16	-0.0758
SMWvp62	-0.1762	WCr7	0.0223	ADk43	-0.0599	SIs18	-0.0666
SIs12	-0.1573	CEp29	0.0307	CDsb52	-0.0447	WCr9	-0.0535
CEs27	-0.1328	ADp35	0.0945	ADp39	-0.0319	SIs20	-0.0382
RCvp50	-0.1143	ADk40	0.1081	ADp35	-0.0278	ADk45	-0.0058
RCk49	-0.0711	ADk33	0.136	SIs20	-0.0128	WCr10	-0.0039
CPvp54	-0.0601	BCHs5	0.1508	SIs21	-0.0117	B/GHp3	0.0388
CPvp53	-0.0333	ADk34	0.1922	ADp36	0.0183	ADp35	0.0596
RCk48	-0.0236	ADk42	0.2051	SIs11	0.0192	ADp38	0.0737
B/GHp2	-0.0061	SIs20	0.2114	ADp38	0.0192	CEs27	0.1088
CEs28	0.0238	B/GHp1	0.2297	CEs27	0.0362	ADk34	0.1192
B/GHp3	0.1163	CEs28	0.2353	ADk33	0.0416	B/GHp2	0.1442
BCHs6	0.1728	BCHs6	0.2574	ADk34	0.0546	ADk33	0.1463
ADk41	0.2489	CEs27	0.2593	CEp30	0.0585	RCs46	0.1502
BCHs5	0.3452	SIs25	0.2931	SIs12	0.0799	RCs47	0.1615
B/GHp1	0.3847	SIs23	0.2956	ADk41	0.0841	BHHk4	0.1687
ADp39	0.6658	SIs26	0.3942	ADk42	0.096	ADp37	0.1899
ADk40	0.7109	SIs19	0.4088	CEp29	0.1879	ADk42	0.1956
ADk42	0.7866	BHHk4	0.4468	CEsb32	0.1982	ADp39	0.2376
ADp37	0.8204	B/GHp3	0.4768	BHHk4	0.2512	CEs28	0.2434
WCr7	0.8792	SIs14	0.4773	BCHs5	0.2686	Ssb65	0.2492
WCr9	0.9119	SIs12	0.4852	CPvd55	0.2822	Ssb64	0.2768
ADk43	0.9185	SIs22	0.5052	CEs28	0.2838	ADk41	0.2958
ADk33	0.9351	SIs17	0.571	BCHs6	0.3403	CDsb52	0.2993
ADk44	0.9351	SIs15	0.5834	B/GHp1	0.3855	RCvp51	0.3064
ADk34	0.9364	SIs13	0.5903	B/GHp3	0.449	BCHs5	0.3349
ADp38	0.9409	SIs11	0.5955	B/GHp2	0.5287	BCHs6	0.3612
ADp35	0.958	SIs16	0.6009	CPvp53	0.5395	CEsb32	0.469
WCr8	0.9657	SIs21	0.6443	CPvp54	0.6722	CEp29	0.4718
ADp36	1.053	SIs24	0.649	CPv56	0.7951	B/GHp1	0.5175
WCr10	1.0704	B/GHp2	0.7128	CPsb58	1.025	CEsb31	0.6296
ADk45	1.1518	SIs18	0.8071	CPsb57	1.162	CEp30	0.6367

Table S11: Factor scores for the PCA analysis of organohalogens in wild bird eggs from South Africa

EIG	*0.3371*	*EIG*	*0.1715*	*EIG*	*0.1148*	*EIG*	*0.0675*
NAME	**AX1**	**NAME**	**AX2**	**NAME**	**AX3**	**NAME**	**AX4**
BDE-208	-1.4712	CB101	-1.7469	CB31	-1.8944	CB149	-2.2326
BDE-207	-1.4572	α-HC H	-1.687	CB74	-1.5641	BDE-47	-1.8346
BDE-209	-1.3532	tr_chl	-1.6168	CB28	-1.322	oxy_ cl	-1.4855
BDE-99	-1.3374	cis- ncl	-1.5873	HBCD	-1.0986	cis- cl	-1.378
BDE-206	-1.2241	cis- cl	-1.5576	CB101	-1.0631	γ-HC H	-1.3681
HBCD	-1.1228	CB52	-1.5153	CB141	-0.9718	tr_ncl	-1.3462
BDE-183	-1.0936	BDE-47	-1.4051	CB151	-0.89	CB128	-1.2994
CB47	-1.0097	CB56	-1.3767	CB52	-0.877	CB66	-1.2441
BDE-153	-0.9336	HCB	-1.3229	γ-HC H	-0.8748	CB137	-1.1366
BDE-28	-0.8407	BDE-28	-1.1527	CB123	-0.8609	CB99	-1.047
tr_ncl	-0.7909	CB110	-1.0765	BDE-47	-0.8314	cis- ncl	-1.0204
tr_chl	-0.7106	CB47	-0.9571	BDE-154	-0.8087	CB105	-1.0001
p.p. -DDT	-0.6312	BDE-99	-0.945	CB110	-0.8001	CB187	-0.9033
CB52	-0.5406	CB66	-0.8329	CB105	-0.7892	CB101	-0.815
HCB	-0.4292	γ-HC H	-0.7932	CB47	-0.7246	CB141	-0.635
CB209	-0.3886	Mire x	-0.6359	p.p. -DDE	-0.7156	CB28	-0.5397
CB56	-0.38	CB149	-0.5197	CB66	-0.7155	BDE-208	-0.5183
cis- cl	-0.2671	oxy_ cl	-0.4929	CB128	-0.6186	CB110	-0.5177
BDE-47	-0.2371	CB137	-0.4506	BDE-100	-0.5606	BDE-100	-0.5162
Mire x	-0.1652	CB151	-0.4483	BDE-209	-0.5001	BDE-207	-0.4848
CB149	-0.0313	β-HC H	-0.376	BDE-99	-0.4852	BDE-153	-0.3682
CB101	0.0263	CB141	-0.1652	BDE-207	-0.4706	CB138	-0.3409
p.p. -DDD	0.0342	tr_ncl	-0.1587	BDE-208	-0.4638	BDE-183	-0.3172
cis- ncl	0.111	CB28	-0.134	CB99	-0.342	CB114	-0.3037
CB110	0.1131	CB167	-0.0765	BDE-206	-0.3052	CB74	-0.2404
CB141	0.1341	CB99	-0.0396	α-HC H	-0.3044	tr_chl	-0.0759
CB151	0.1426	CB123	-0.0173	CB149	-0.1991	CB123	-0.0755
γ-HC H	0.178	CB31	-0.0049	CB56	-0.1672	CB183	-0.005
oxy_ cl	0.2103	p.p. -DDD	0.0335	CB138	-0.0627	CB47	0.0235
BDE-154	0.3064	BDE-100	0.0398	CB114	-0.0579	CB170	0.0329
α-HC H	0.3515	CB118	0.0451	CB137	0.0008	CB157	0.0712
BDE-100	0.5232	CB199	0.0697	BDE-153	0.0038	CB156	0.1231
CB206	0.5278	CB74	0.2317	BDE-28	0.0041	β-HC H	0.2895
CB31	0.5514	HBCD	0.31	β-HC H	0.0429	HBCD	0.2983
CB196	0.7337	CB138	0.3435	CB156	0.0637	BDE-154	0.356
CB137	0.7502	BDE-209	0.3758	CB157	0.1661	BDE-206	0.3753
CB194	0.8253	CB128	0.3776	BDE-183	0.2483	HCB	0.3875
CB199	0.8406	CB209	0.4164	CB170	0.2874	p.p. -DDD	0.5083
CB66	0.8998	p.p. -DDE	0.4234	CB118	0.3819	CB196	0.5353
CB118	0.9825	CB114	0.4251	CB167	0.4363	p.p. -DDT	0.5862
CB28	0.9899	BDE-206	0.4397	tr_chl	0.4848	CB180	0.5921
CB189	1.0109	CB105	0.4421	CB153	0.5013	CB151	0.6104
β-HC H	1.0974	CB153	0.4438	CB180	0.654	CB31	0.6374
CB123	1.1259	CB157	0.4751	CB183	0.6696	BDE-99	0.6985
p.p. -DDE	1.146	CB187	0.4788	p.p. -DDT	0.6869	CB118	0.7768
CB128	1.1469	BDE-154	0.6161	CB199	0.7699	CB153	0.7926
CB187	1.1502	BDE-208	0.9072	CB187	0.7977	CB206	0.8723
CB74	1.217	CB156	0.9486	p.p. -DDD	0.8203	CB52	0.9948
CB183	1.2941	CB189	1.0282	CB189	1.044	CB189	1.0534
CB114	1.3113	BDE-207	1.0407	cis- cl	1.1867	BDE-209	1.0579
CB180	1.3217	CB194	1.0699	CB196	1.2177	CB167	1.097
CB153	1.3257	CB183	1.0797	CB194	1.3337	CB194	1.1804
CB156	1.3779	CB170	1.1227	CB206	1.36	α-HC H	1.2036
CB170	1.3785	CB180	1.1999	CB209	1.3804	CB199	1.3566
CB105	1.3902	p.p. -DDT	1.2783	HCB	1.6169	p.p. -DDE	1.6116
CB99	1.414	CB196	1.4324	cis- ncl	1.7652	CB209	1.8813
CB138	1.4296	CB206	1.4721	Mire x	1.7771	Mire x	1.9862
CB167	1.4472	BDE-153	1.6561	oxy_ cl	2.2387	BDE-28	2.0003
CB157	1.5333	BDE-183	1.6954	tr_ncl	2.2939	CB56	2.4214

Table S12: Ratio's of DDE:ΣPCBs and ΣPBDEs:ΣPCBs organohalogens in wild bird eggs

Species	Guild	Eco-system	Sampling area	Ratio DDE:PCB	Ratio PBDE:PCB
African Sacred Ibis	Scavenger	Combined	Soweto	0.73	0.75
African Sacred Ibis	Scavenger	Combined	Soweto	0.78	0.52
African Sacred Ibis	Scavenger	Combined	Soweto	1.43	0.87
African Sacred Ibis	Scavenger	Combined	Soweto	0.33	0.55
African Sacred Ibis	Scavenger	Combined	Soweto	1.29	1.07
African Sacred Ibis	Scavenger	Combined	Soweto	0.54	0.66
African Sacred Ibis	Scavenger	Combined	Soweto	0.34	0.76
African Sacred Ibis	Scavenger	Combined	Soweto	1.02	7.26
African Sacred Ibis	Scavenger	Combined	Soweto	0.95	1.08
African Sacred Ibis	Scavenger	Combined	Soweto	0.19	0.17
African Sacred Ibis	Scavenger	Combined	Soweto	0.67	0.84
African Sacred Ibis	Scavenger	Combined	Soweto	0.81	0.40
African Sacred Ibis	Scavenger	Combined	Soweto	0.52	0.44
African Sacred Ibis	Scavenger	Combined	Soweto	0.41	0.74
African Sacred Ibis	Scavenger	Combined	Soweto	0.57	0.52
African Sacred Ibis	Scavenger	Combined	Soweto	0.39	0.42
African Sacred Ibis	**Scavenger**	**Combined**	**Soweto**	**0.62**	**0.70**
Cattle Egret	Omnivores/Insectivores	Terestrial	Soweto	0.93	0.15
Cattle Egret	Omnivores/Insectivores	Terestrial	Soweto	0.99	0.10
Cattle Egret	**Omnivores/Insectivores**	**Terestrial**	**Soweto**	**0.96**	**0.13**
Cattle Egret	Omnivores/Insectivores	Terestrial	Parys	1.44	0.41
Cattle Egret	Omnivores/Insectivores	Terestrial	Parys	4.65	5.15
Cattle Egret	**Omnivores/Insectivores**	**Terestrial**	**Parys**	**3.04**	**2.78**
Cattle Egret	Omnivores/Insectivores	Terestrial	Sasolburg	3.00	6.00
Cattle Egret	Omnivores/Insectivores	Terestrial	Sasolburg	0.72	0.51
Cattle Egret	**Omnivores/Insectivores**	**Terestrial**	**Sasolburg**	**1.86**	**3.25**
Cattle Egret	**Omnivores/Insectivores**	**Terestrial**	**Mixed**	**1.21**	**0.46**
African Darter	Piscivores	Aquatic	Kempton Park	11.25	0.02
African Darter	Piscivores	Aquatic	Kempton Park	0.89	0.02
African Darter	Piscivores	Aquatic	Kempton Park	0.65	0.03
African Darter	Piscivores	Aquatic	Kempton Park	2.52	0.20
African Darter	Piscivores	Aquatic	Kempton Park	1.45	0.02
African Darter	Piscivores	Aquatic	Kempton Park	1.12	0.02
African Darter	Piscivores	Aquatic	Kempton Park	1.41	0.02
African Darter	Piscivores	Aquatic	Kempton Park	2.59	0.01
African Darter	**Piscivores**	**Aquatic**	**Kempton Park**	**1.43**	**0.02**
African Darter	Piscivores	Aquatic	Parys	0.61	0.02
African Darter	Piscivores	Aquatic	Parys	0.67	0.01
African Darter	Piscivores	Aquatic	Parys	1.83	0.03
African Darter	Piscivores	Aquatic	Parys	1.18	0.02
African Darter	Piscivores	Aquatic	Parys	1.01	0.06
African Darter	**Piscivores**	**Aquatic**	**Parys**	**1.01**	**0.02**
African Darter	**Piscivores**	**Aquatic**	**Mixed**	1.18	0.02
White-breasted Cormorant	Piscivores	Aquatic	Roodeplaat Dam	2.41	0.02
White-breasted Cormorant	Piscivores	Aquatic	Roodeplaat Dam	2.10	0.02
White-breasted Cormorant	Piscivores	Aquatic	Roodeplaat Dam	1.70	0.03
White-breasted Cormorant	Piscivores	Aquatic	Roodeplaat Dam	2.22	0.01
White-breasted Cormorant	**Piscivores**	**Aquatic**	**Roodeplaat Dam**	1.57	0.02
Heron *sp*	Piscivores	Aquatic	Soweto	2.23	0.03
Heron *sp*	Piscivores	Aquatic	Soweto	2.76	0.04
Heron sp	**Piscivores**	**Aquatic**	**Soweto**	**2.50**	**0.03**
Heron *sp*	**Piscivores**	**Aquatic**	**Kempton Park**	**3.86**	**1.15**
Heron *sp*	Piscivores	Aquatic	Parys	2.76	0.05
Heron *sp*	Piscivores	Aquatic	Parys	1.73	0.33
Heron *sp*	Piscivores	Aquatic	Parys	0.38	0.19
Heron sp	**Piscivores**	**Aquatic**	**Parys**	**1.73**	**0.19**
Heron sp	**Piscivores**	**Aquatic**	**Mixed**	**2.50**	**0.12**
Crowned Lapwing	Omnivores/Insectivores	Terestrial	Vanderbijlpark	0.20	0.07
Crowned Lapwing	Omnivores/Insectivores	Terestrial	Vanderbijlpark	0.08	0.07
Crowned Lapwing	Omnivores/Insectivores	Terestrial	Vanderbijlpark	0.10	0.44
Crowned Lapwing	Omnivores/Insectivores	Terestrial	Vanderbijlpark	0.10	0.25
Crowned Lapwing	**Omnivores/Insectivores**	**Terestrial**	**Vanderbijlpark**	**0.10**	**0.16**
Crowned Lapwing	Omnivores/Insectivores	Terestrial	Sasolburg	0.06	0.09
Crowned Lapwing	Omnivores/Insectivores	Terestrial	Sasolburg	0.23	0.12
Crowned Lapwing	**Omnivores/Insectivores**	**Terestrial**	**Sasolburg**	**0.15**	**0.10**
Crowned Lapwing	**Omnivores/Insectivores**	**Terestrial**	**Sasolburg**	**0.10**	**0.10**
Red-knobbed Coot	Omnivores/Insectivores	Aquatic	Soweto	1.08	1.25
Red-knobbed Coot	Omnivores/Insectivores	Aquatic	Soweto	0.98	26.87
Red-knobbed Coot	**Omnivores/Insectivores**	**Aquatic**	**Soweto**	**1.03**	**14.06**
Red-knobbed Coot	Omnivores/Insectivores	Aquatic	Kempton Park	2.90	0.30
Red-knobbed Coot	Omnivores/Insectivores	Aquatic	Kempton Park	3.63	0.38
Red-knobbed Coot	**Omnivores/Insectivores**	**Aquatic**	**Kempton Park**	**3.26**	**0.34**
Red-knobbed Coot	Omnivores/Insectivores	Aquatic	Vanderbijlpark	2.78	0.26
Red-knobbed Coot	Omnivores/Insectivores	Aquatic	Vanderbijlpark	0.87	2.34
Red-knobbed Coot	**Omnivores/Insectivores**	**Aquatic**	**Vanderbijlpark**	**1.82**	**1.30**
Red-knobbed Coot	**Omnivores/Insectivores**	**Aquatic**	**Mixed**	**1.93**	**0.82**
Granivore	Granivore	Terestrial	Sasolburg	3.14	14.04
Granivore	Granivore	Terestrial	Sasolburg	0.43	2.17
Granivore	Granivore	Terestrial	Sasolburg	0.03	0.83
Granivore	**Granivore**	**Terestrial**	**Sasolburg**	**0.43**	**2.17**
Granivore	**Granivore**	**Terestrial**	**Parys**	**1.26**	**0.72**
Granivore	Granivore	Terestrial	Vanderbijlpark	0.45	24.48
Granivore	Granivore	Terestrial	Vanderbijlpark	0.57	0.48
Granivore	**Granivore**	**Terestrial**	**Vanderbijlpark**	**0.51**	**12.48**
Granivore	Granivore	Terestrial	Soweto	0.65	0.40
Granivore	Granivore	Terestrial	Soweto	0.35	1.73
Granivore	**Granivore**	**Terestrial**	**Soweto**	**0.50**	**1.07**
Granivore	**Granivore**	**Terestrial**	**Mixed**	**0.51**	**1.28**

Table S13: Egg parameters used to determine the Ratcliffe index

Samlpe	Average mass (g)	Cifcum-ference (cm)	Length (cm)	Mass of homo-genate (g)	Mass of shell (g)	Sanple	Average mass (g)	Cifcum-ference (cm)	Length (cm)	Mass of homo-genate (g)	Mass of shell (g)
B/GHp1	47.57	14.00	16.90	40.63	6.94	**CEsb31**	24.62	13.50	11.20	21.03	3.59
B/GHp2	61.48	15.10	17.60	50.86	10.62	**CEsb32**	26.82	11.00	13.70	14.40	12.42
B/GHp3	64.37	15.40	17.40	52.89	11.48	**ADk33**	32.55	11.80	14.80	27.64	4.91
BHHk4	27.29	12.00	13.00	22.38	4.91	**ADk34**	39.15	12.50	15.70	32.77	6.38
BCHs5	26.66	12.10	13.30	20.50	6.16	**ADp35**	31.18	12.80	14.60	28.07	3.11
BCHs6	24.98	11.90	13.50	19.40	5.58	**ADp36**	29.70	11.60	14.60	25.01	4.69
WCr7	55.80	14.10	18.40	45.94	9.86	**ADp37**	41.41	12.30	16.40	34.20	7.21
WCr8	44.85	13.00	17.10	36.74	8.11	**ADp38**	32.25	12.30	15.10	25.42	6.83
WCr9	46.85	12.90	17.00	37.43	9.42	**ADp39**	29.27	12.10	15.00	24.62	4.65
WCr10	62.27	13.80	18.40	52.89	9.38	**ADk40**	35.32	12.20	14.70	28.12	7.20
SIs11	60.48	14.50	18.00	51.99	8.49	**ADk41**	31.70	12.30	15.00	26.67	5.03
SIs12	49.73	13.40	16.60	42.62	7.11	**ADk42**	32.01	11.20	14.50	26.38	5.63
SIs13	63.33	14.50	18.20	53.50	9.83	**ADk43**	38.02	12.50	15.40	31.27	6.75
SIs14	55.37	13.70	16.70	43.98	11.39	**ADk44**	31.65	12.10	14.80	26.75	4.90
SIs15	65.53	15.30	17.70	55.56	9.97	**ADk45**	37.18	13.00	14.60	30.61	6.57
SIs16	57.70	13.90	17.40	46.22	11.48	**RCs46**	42.10	12.70	16.00	34.10	8.00
SIs17	58.18	14.10	17.90	45.59	12.59	**RCs47**	40.28	12.20	15.70	34.12	6.16
SIs18	56.02	13.50	17.50	44.81	11.21	**RCk48**	45.09	13.50	16.10	35.23	9.86
SIs19	56.10	14.00	17.10	45.82	10.28	**RCk49**	45.60	13.20	16.40	38.42	7.18
SIs20	63.32	15.00	18.00	50.77	12.55	**RCvp50**	44.43	13.20	15.50	37.27	7.16
SIs21	60.48	14.20	17.50	50.48	10.00	**RCvp51**	24.25	12.00	13.10	16.95	7.30
SIs22	49.07	13.60	16.10	41.08	7.99	**CPvp53**	17.16	11.40	12.90	13.16	4.00
SIs23	63.39	14.50	17.50	50.90	12.49	**CPvp54**	14.02	9.00	10.90	11.23	2.79
SIs24	48.06	13.90	16.00	40.30	7.76	**CPvp55**	14.49	10.60	11.80	11.99	2.50
SIs25	55.67	13.10	18.10	47.17	8.50	**CPvp56**	16.83	9.80	12.20	12.68	4.15
SIs26	53.98	13.50	17.80	44.52	9.46	**CPsb57**	18.54	10.40	11.80	14.68	3.86
CEs27	24.32	10.80	13.40	20.74	3.58	**CPsb58**	15.51	9.80	11.70	12.69	2.82
CEs28	28.12	11.40	14.30	22.22	5.90						
CEp29	26.76	11.50	13.10	22.99	3.77						
CEp30	26.99	12.00	13.40	21.06	5.93						

Table S14: Ratcliffe index for both historical and current data

Species	Year	Ratcliffe Index	Reference	Species	Year	Ratcliffe Index	Reference
African fish eagle	1989	2.45	Davies & Randall, 1989	Cape Vulture eggs	1982	4.25	Mundy *et al*., 1982
African fish eagle	1989	2.70		Cape Vulture eggs	1982	3.63	
African fish eagle	1989	2.66		Cape Vulture eggs	1982	4.12	
African fish eagle	1989	2.77		Cape Vulture eggs	1982	3.64	
African fish eagle	1989	2.72		Cape Vulture eggs	1982	3.52	
African fish eagle	1989	2.50		Cape Vulture eggs	1982	3.85	
African fish eagle	1989	2.13		Cape Vulture eggs	1982	3.79	
African fish eagle	1989	2.02		Cape Vulture eggs	1982	3.22	
African fish eagle	1989	2.39		Cape Vulture eggs	1982	4.05	
African fish eagle	1989	2.15		Cape Vulture eggs	1982	3.96	
African fish eagle	1989	2.22		B/GHp1	2008	1.83	Current study
African fish eagle	1989	2.22		B/GHp2	2008	2.50	
African fish eagle	1989	2.47		B/GHp3	2008	2.68	
African fish eagle	1989	3.05		BHHk4	2008	1.97	
African fish eagle	1989	2.59		BCHs5	2008	2.39	
African fish eagle	1989	2.40		BCHs6	2008	2.17	
African fish eagle	1989	2.70		WCr7	2008	2.38	Current study
African fish eagle	1989	2.62		WCr8	2008	2.28	
Lanner falcon	1984	1.57	Snelling *et al*., 1984	WCr9	2008	2.68	
Lanner falcon	1984	1.59		WCr10	2008	2.31	
Lanner falcon	1984	1.56		SIs11	2008	2.03	Current study
Lanner falcon	1984	1.52		SIs12	2008	2.00	
Lanner falcon	1984	1.86		SIs13	2008	2.33	
Lanner falcon	1984	1.72		SIs14	2008	3.11	
Lanner falcon	1984	1.81		SIs15	2008	2.30	
Lanner falcon	1984	1.36		SIs16	2008	2.97	
Lanner falcon	1984	1.68		SIs17	2008	3.12	
Lanner falcon	1984	1.63		SIs18	2008	2.96	
Lanner falcon	1984	1.39		SIs19	2008	2.68	
Black Sparrowhawk	1984	2.05	Snelling *et al*., 1984	SIs20	2008	2.90	
Black Sparrowhawk	1984	1.91		SIs21	2008	2.52	
Black Sparrowhawk	1984	1.88		SIs22	2008	2.28	
Black Sparrowhawk	1984	2.34		SIs23	2008	3.08	
Black Sparrowhawk	1984	2.35		SIs24	2008	2.18	
Black Sparrowhawk	1984	2.13		SIs25	2008	2.24	
Black Sparrowhawk	1984	2.33		SIs26	2008	2.46	
Black Sparrowhawk	1984	2.20		CEs27	2008	1.54	Current study
Black Sparrowhawk	1984	1.96		CEs28	2008	2.26	
Black Sparrowhawk	1984	1.77		CEp29	2008	1.56	
Black Sparrowhawk	1984	1.92		CEp30	2008	2.30	
Black Sparrowhawk	1984	1.85		CEsb31	2008	1.48	
Black Sparrowhawk	1984	1.86		CEsb32	2008	5.15	
Black Sparrowhawk	1984	1.76		ADk33	2008	1.76	Current study
Black Sparrowhawk	1984	2.04		ADk34	2008	2.03	
Black Sparrowhawk	1984	1.54		ADp35	2008	1.04	
Black Sparrowhawk	1984	1.82		ADp36	2008	1.73	
Black Sparrowhawk	1984	1.50		ADp37	2008	2.23	
Black Sparrowhawk	1984	1.89		ADp38	2008	2.30	
Black Sparrowhawk	1984	2.07		ADp39	2008	1.60	
Black Sparrowhawk	1984	1.85		ADk40	2008	2.51	
Bateleur	1984	2.56	Snelling *et al*., 1984	ADk41	2008	1.71	
Bateleur	1984	2.99		ADk42	2008	2.17	
Cape Vulture eggs	1982	3.55	Mundy *et al*., 1982	ADk43	2008	2.19	
Cape Vulture eggs	1982	4.09		ADk44	2008	1.71	
Cape Vulture eggs	1982	3.82		ADk45	2008	2.16	
Cape Vulture eggs	1982	3.89		RCs46	2008	2.46	Current study
Cape Vulture eggs	1982	3.81		RCs47	2008	2.01	
Cape Vulture eggs	1982	3.65		RCk48	2008	2.84	
Cape Vulture eggs	1982	4.31		RCk49	2008	2.07	
Cape Vulture eggs	1982	3.70		RCvp50	2008	2.19	
Cape Vulture eggs	1982	4.22		RCvp51	2008	2.90	
Cape Vulture eggs	1982	3.51		CPvp53	2008	1.70	Current study
Cape Vulture eggs	1982	4.17		CPvp54	2008	1.78	
Cape Vulture eggs	1982	4.08		CPvp55	2008	1.25	
Cape Vulture eggs	1982	3.51		CPvp56	2008	2.17	
Cape Vulture eggs	1982	3.62		CPsb57	2008	1.97	
Cape Vulture eggs	1982	3.67		CPsb58	2008	1.54	