

PERSISTENT ORGANIC POLLUTANT LEVELS IN MAGNIFICENT FRIGATEBIRD *FREGATA MAGNIFICENS* IN SOUTHEASTERN BRAZIL

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SUMMARY

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This article reports the findings of analyses of toxic contaminants in Magnificent Frigatebird *Fregata magnificens*, a fish-eating seabird that has a high trophic position and a low ability to metabolise xenobiotic compounds. Frigatebirds were collected at Ilha Grande Bay, Rio de Janeiro state, Brazil (23°8'26"S, 44°14'50"W) between February 2009 and April 2013. Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) were identified and quantified in tissue composites using gas chromatography/mass spectrometry analyses. The concentrations found were below the range of concern established by the World Health Organization. The sum of analysed PCB-congeners had concentrations of 968 pg/g and 13.9 pg toxic equivalence (TEQ)/g lipid weight. The PCDD/PCDF-congeners had concentrations of 331.04 pg/g and 13.4 pg TEQ/g lipid weight. This study provides information from analyses of seabirds concerning halogenated hydrocarbons as scientific support for policy-makers regarding the management of the State of Rio de Janeiro's coastal zone.

Keywords: Ilha Grande Bay, halogenated hydrocarbons, *Fregata magnificens*, contamination, anthropic actions, sustainability

INTRODUCTION

Halogenated aromatic compounds such as polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are industrial byproducts found widely in the environment and in chemical-waste dumpsites (Lauwerys & Hoet 1993). Halogenated aromatics are present in a variety of analytes as highly complex mixtures of isomers and congeners, complicating the detection and risk assessment of these compounds (Safe 1990). They persist in the environment (Xu *et al.* 2013) and bioaccumulate in living organisms, preferentially in lipids or fatty tissues (Parera *et al.* 2013, Tanabe *et al.* 2004).

Due to the strength of the carbon-chlorine bond, they tend to be resistant to common degradation pathways, and subsequently have an environmental half-life of years to decades (Dorneles *et al.* 2013). This persistence in the environment increases the concentration of these compounds throughout the food chain, ultimately leading to toxic effects, especially at the highest trophic levels (Kumar *et al.* 2002, Moriarty 1999). It also allows these compounds to be transported over long distances, either through the atmosphere or by ocean currents, depending on the physical properties of the molecule (Alcock *et al.* 1998). Many of these compounds were developed as agricultural pesticides (e.g. DDT [dichlorodiphenyltrichloroethane], Chlordane [hexachlorocyclopentadiene] and Lindane [gamma-hexachlorocyclohexane]) or for use in industrial applications (e.g. PCBs and brominated flame retardants), while others are byproducts of industrial synthesis or incomplete combustion (e.g. PCDDs) (Allen *et al.* 2008). Although some of these compounds are known to be toxic, they are still in use today. For instance, DDT is still used for malaria control in Africa (Ranson *et al.* 2011) and as an anti-fouling agent in paint in China (Lin *et al.* 2009). As a result, in 2007 the United Nations Environmental Programme (UNEP

2007) estimated that 250 t of DDT are released to the environment every year. In addition, the increasing use of pesticides and PCBs in agriculture, and their presence in agricultural drainage systems, represents the most dangerous pollution of the environment with these compounds (García de la Parra *et al.* 2012).

Organochlorine compounds reach the marine environment through wet and dry surface water deposition and diffusive vapour exchange between air and water (Wania *et al.* 1998, Ferreira 2008). Transport from sediment to water is of great concern. Previously polluted sediments may act as a source of toxic compounds to the overlying water column, prolonging the exposure of biota long after emissions have stopped. The key processes that determine the transport of organochlorines over the sediment-water interface are (a) the sedimentation and resuspension of particles, (b) the diffusive movement of organochlorine compounds and (c) their attachment to dissolved organic matter (Holmström & Berger 2008).

Seabirds are good indicators of productivity and health in the marine environment (Parrish & Zador 2003, Tasker & Reid 1997). Their patterns of distribution and abundance are strongly correlated with primary production (Whitehouse *et al.* 1999), abundance of fish shoals (Frederiksen *et al.* 2008) and nest-site availability (Fasola & Canova 1992, Bourgeois *et al.* 2008). Population fluctuations, mass mortality and other phenomena affecting populations can be used to identify the presence of contaminants in the sea (Kushlan 1993). Therefore, seabirds have been used in several environmental-monitoring studies (Choy *et al.* 2010, Lauwerys & Hoet 1993).

One potentially useful species in this regard is the Magnificent Frigatebird *Fregata magnificens*, since it occurs at the top of the food web. This species occurs along the tropical and subtropical coasts of the Americas (Harrison 1983), including Brazil, where

colonies are found on the island of Fernando de Noronha and in the states of Bahia, Rio de Janeiro, São Paulo, Paraná and Santa Catarina (Alves & Vecchi 2009, Coelho *et al.* 1991).

The present work aimed to evaluate the concentrations of PCDD/PCDFs and PCBs in *F. magnificens* that nest at Ilha Grande Bay, Rio de Janeiro state, Brazil.

METHODS

Study site

The Ilha Grande Bay is a large area comprising coastline, waters and an island in Rio de Janeiro state, Brazil (Alho *et al.* 2002). It is characterised by a system of estuarine and oligotrophic waters with a significant diversity of marine ecosystem that includes rocky shores, islands, mangroves and sandy beaches (Fig. 1).

Sample collection and handling

Dead or injured adult frigatebirds ($n = 17$) were collected from Ilha Grande Bay ($23^{\circ}8'26''S$, $44^{\circ}14'50''W$) between February 2009 and April 2013. After collection, an external assessment of the birds was followed by necropsy, according to a standard protocol (Jauniaux *et al.* 1998). Livers were collected, weighed and kept frozen until the time of analysis.

Analysis of dioxin, furan and PCB congeners

The analytical procedure was described by Kumar *et al.* (2001), Parvez *et al.* (2013) and Shaw *et al.* (2006). Moisture content was determined and samples were extracted using a Soxhlet apparatus for 10–15 h in dichloromethane. Briefly, after the extraction, samples were concentrated to 10 mL using a Kuderna-Danish (K-D) concentrator, and the solvent was transferred to n-hexane. Lipid content was determined gravimetrically from an aliquot of the extract. Seventeen ^{13}C -labelled 2,3,7,8-substituted tetra-, penta-, hexa-, hepta- and octa-CDD and CDF congeners and 12 dioxin-like PCBs (IUPAC Nos. 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167 and 189) were spiked into hexane extracts before treatment with sulphuric acid. The hexane layer was rinsed twice with hexane-washed water and dried by passing through anhydrous sodium

sulphate in a glass funnel. The solution was concentrated to 2 mL and sequentially subjected to silica gel, alumina and silica gel-impregnated activated carbon column chromatography. Extracts were passed through activated silica gel packed in a glass column (Wakogel, silica gel 60; 2 g) and eluted with 130 mL of hexane, which contained PCDD/PCDFs and dioxin-like PCBs. The hexane extract was K-D concentrated, passed through activated alumina column (Merck-Alumina oxide, activity grade 1; 5g) and eluted with 30 mL of 2% dichloromethane in hexane as the first fraction, which contained several *ortho*-substituted PCBs. The second fraction was eluted with 30 mL of 50% dichloromethane in hexane contained PCDD/PCDFs and dioxin-like PCBs, which was purged under a gentle stream of nitrogen to 0.5 mL and passed through silica gel impregnated activated carbon column (0.5 g) to further separate mono- and di-*ortho* PCBs from non-*ortho* PCBs and PCDD/PCDFs. The first fraction eluted with 25 mL of 25% dichloromethane in hexane contained mono and di-*ortho* PCBs. The second fraction, eluted with 250 mL toluene, contained non-*ortho* PCBs and PCDD/PCDFs. Sample extracts were analysed using a high-resolution gas chromatograph interfaced with a high-resolution mass spectrometer (HRGC-HRMS). Procedural blanks ($n = 3$) were analysed to check for interferences. Heptachlorinated dibenzo-*p*-dioxin (HpCDD) and heptachlorodibenzofuran (HpCDF) were detected in blanks at concentrations of approximately <0.01 pg/g and octachlorodibenzo-*p*-dioxin (OCDD) was detected at a concentration of approximately 0.1 pg/g. The values obtained for HpCDD, OCDD and HpCDF were not corrected for the blank concentrations.

Identification and quantification

Identification and quantification of 2378-substituted congeners of PCDD/PCDFs and dioxin-like PCBs were performed using gas chromatography followed by high-performance liquid chromatography. Gas chromatography: Shimadzu GC-14B gas chromatograph with AOC-1400 auto-sampler. Columns: CBP-1 (SE-30) and CBP-5 (SE-52/54 confirmatory column). Injection: Splitless (30 s) 300 °C. Temperature program of the oven: 110 °C (1 minute); 15 °C/min up to 170 °C; 7.5 °C/min up to 290 °C; hold for 10 minutes. Total run time: 25 minutes. Electron capture detector (^{63}Ni) temperature: 310 °C. HPLC: Shimadzu LC-10AS; Mobile phase: acetonitrile: water 80%, isocratic run. Column: Shimadzu STR-ODS-II (C18 reversed phase) 25 cm, L: 4 mm ID. UV/VIS detector model: Shimadzu SPD-10A. Prior to injection, ^{13}C -labelled 1234-TeCDD and 123789-HxCDD were added as injection recovery standard. The mean recovery of spiked internal standard through the whole analytical procedure was 74% (range: 60%–95%), and the reported concentrations were not corrected for the surrogate recoveries. PCDD/PCDFs, dioxin-like PCBs and TEQ concentrations are reported on a lipid weight basis using the Toxic Equivalency Factor published by the World Health Organization (WHO-TEF) for birds (Van den Berg *et al.*, 2006) as pg TEQ/g lipid weight.

Statistical tests

The hypothesis of normal distribution (Shapiro-Wilk's *W* test) was not rejected for log-transformed data. Lipid base log-transformed concentrations were used to determine significant differences between group geometric means (Tukey test). The null hypothesis (equality of means) was rejected at the 95% significance level ($P < 0.05$).

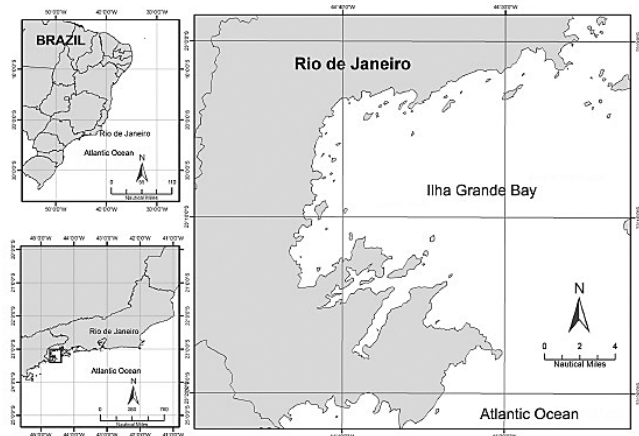


Fig. 1. Map of the study area: Ilha Grande Bay, Rio de Janeiro State, southeastern Brazil.

RESULTS

PCDD, PCDF and PCB congeners

All samples analysed ($n = 17$) contained 2,3,7,8-substituted PCDD and PCDF congeners (Table 1). No significant sex-related

TABLE 1
Concentrations of halogenated aromatic compounds found in *Fregata magnificens*

Compounds	Median (range) concentration, pg/g lipid weight	Toxic equivalent from WHO-TEF (birds), pg TEQ/g lipid weight
<i>Dibenzo-p-dioxins (PCDD)</i>		
2378-TCDD	0.6 (0.2–4)	0.6
12378-PeCDD	3 (0.5–8)	3
123478-HxCDD	14 (6–44)	0.7
123678-HxCDD	5 (1–9)	0.05
123789-HxCDD	9 (5–31)	0.9
1234678-HpCDD	35 (6–66)	0.035
OCDD	199 (33–291)	0.0199
<i>Di- and tri-benzofurans (PCDF)</i>		
2378-TCDF	0.34 (1–4)	0.34
12378-PeCDF	16 (9–31)	1.6
23478-PeCDF	4.9 (2–32)	4.9
123478-HxCDF	8.2 (2–32)	0.82
123678-HxCDF	3 (1–12)	0.3
1234789-HxCDF	7 (1–30)	0.7
234678-HxCDF	3 (2–12)	0.3
1234678-HpCDF	9 (3–23)	0.09
1234789-HpCDF	5 (2–22)	0.05
OCDF	9 (2–19)	0.0009
Total	331.04	13.4
<i>Non-ortho PCBs</i>		
3,3',4,4'-TCB (77)	86 (33–543)	4.3
3,4,4',5-TCB (81)	47 (18–550)	4.7
3,3',4,4',5-PeCB (126)	48 (32–111)	4.8
3,3',4,4',5,5'-HxCB (169)	67 (26–104)	0.067
<i>Mono-ortho PCBs</i>		
2,3,3',4,4'-PeCB (105)	244 (37–289)	0.0244
2,3,4,4',5-PeCB (114)	185 (55–312)	0.0185
2,3',4,4',5-PeCB (118)	134 (49–244)	0.00134
2',3,4,4',5-PeCB (123)	61 (22–114)	0.00061
2,3,3',4,4',5-HxCB (156)	18 (7–72)	0.0018
2,3,3',4,4',5'-HxCB (157)	24 (4–33)	0.0024
2,3',4,4',5,5'-HxCB (167)	35 (13–53)	0.00035
2,3,3',4,4',5,5'-HeCB (189)	19 (7–29)	0.00019
Total	968	13.9

differences in PCDD/PCDF or PCB concentrations were found. Fat-based log-transformed concentrations were used to determine whether there were significant differences between group geometric means. The null hypothesis (equality of means) was rejected at the 95% significance level ($P < 0.05$).

There were no statistically significant differences between mean PCDD/PCDF and PCB-congeners concentrations. The five most abundant congeners were 2,3,4,7,8-pentachlorodibenzofuran (PeCDF). In order of abundance, congeners found were 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD), 1,2,3,7,8-pentachlorodibenzofuran (PeCDF), 1,2,3,7,8,9 hexachlorodibenzo-*p*-dioxin (HxCDD) and 2,3,4,6,7,8-hexachlorodibenzofuran (HxCDF). PCB congeners were detected in all samples analysed. The concentration of sum of the 12 PCB congeners analysed was 968 pg/g or 13.9 pg TEQ/g lipid weight. The concentration of the sum of 17 congeners of PCDD and PCDF analysed was 331.04 pg/g and 13.4 pg TEQ/g lipid weight.

Toxic equivalents, calculated using TEFs for birds proposed by the World Health Organization (Van der Berg *et al.* 2006), are shown in Table 1, and compositions are shown in Figure 2. From a toxicological point of view, even if the TEF values for these minor contaminants were as high as PCB or even PCDD values, their contribution to TEQs would be small compared with PCDDs, PCDFs and PCBs. Also, the similarity of the results obtained from the bioassay and the calculation of TEQs indicate that the PCDDs, PCDFs and PCBs analysed account for most of the dioxin-like biological activity observed.

DISCUSSION

Data on contaminant levels in Brazilian seabirds are limited, and no information exists regarding levels of new or emerging contaminants. The responses to high concentrations of PCDD/PCDF and PCB congeners in birds range from adverse effects on reproductive capacity (Custer *et al.* 2010), to direct toxic, teratogenic effects on developing embryos (Harris *et al.*, 2005, Yamashita *et al.* 1993) and to subtle changes in parental behaviour (Harris *et al.* 2005). A comprehensive example of these effects has been shown in a study of fish-eating waterbirds in the northern Baltic (Hario *et al.* 2004).

The predominant compounds found in the present study were PCBs. Similarly, a previous study of frigatebird eggs found PCBs at a concentration of 98.15 ng/g (Trefry *et al.* 2013). This level is

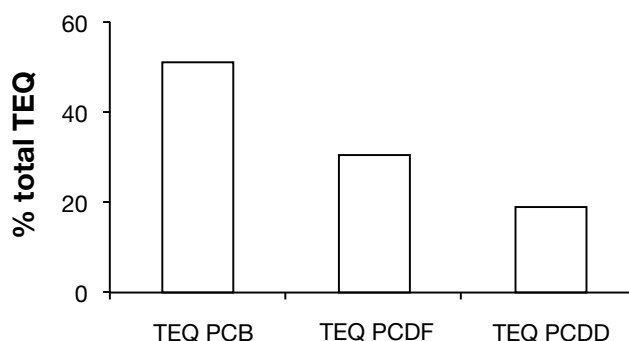


Fig. 2. Contributions of PCDDs, PCDFs and dioxin-like PCBs to total TEQ.

low compared, for example, with those detected in aquatic birds of the Great Lakes region, which average 404 ng/g lipid (Yamashita *et al.* 1993) and in tissues of seabirds that feed near industrialised areas (e.g. near North America), (Tanabe *et al.* 2004). The low levels of contaminants reported in this study suggest a relative degree of isolation and preservation, but the occurrence and distribution profiles of PCBs support the hypothesis that the main source of contamination in remote areas is long-range atmospheric transport and demonstrate the ubiquity of those pollutants in the marine environment.

This study is the first to report concentrations of PCDD/PCDFs and PCBs in seabirds at this site, and the first involving free-ranging birds from the Ilha Grande Bay. Because of the small size of the population studied, continued monitoring of these pollutants is essential in assessing the health and viability of these animals. Biomagnification may play a role in the levels of these pollutants found in the species analysed. Further ecotoxicological impact assessments are recommended on organisms at higher trophic levels. The ubiquity of these pollutants in Ilha Grande Bay's marine environment supports the need for a greater awareness of bioaccumulation processes, particularly in locally farmed and wild shellfish and fish used as food. Additional research on environmental and faunal aspects is needed to better gauge the incidence of chemical contaminants in coastal ecosystems in Brazil.

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