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Chlorinated, brominated and fluorinated organic pollutants in African Penguin eggs: 30 years since the previous assessment

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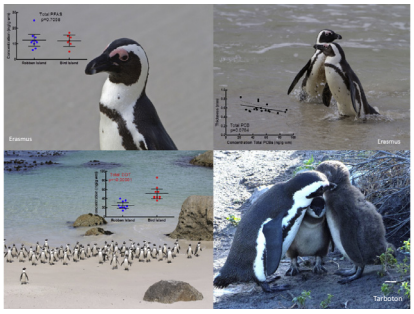
HIGHLIGHTS

- The African Penguin population has crashed, and POPs data are 30 years old.
- ΣPCB concentrations in eggs decreased about four-fold, ΣDDT remained the same.
- Non-significant association between eggshell thickness and ΣDDT and ΣPCB.
- Thinner eggshells poses concern about dehydration in high heat conditions.
- The linkages between pollutants and other effects needs further examination.

GRAPHICAL ABSTRACT



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ABSTRACT

The African Penguin population has drastically declined over the last 100 years. Changes in food availability due to over-fishing and other oceanographic changes seem to be major causes. However, it has also been 30 years since organic pollutants as a potential factor have been assessed. We analysed penguin eggs collected in 2011 and 2012 from two breeding colonies 640 km apart: Robben Island near Cape Town on the Atlantic Ocean coast, and Bird Island near Port Elizabeth on the Indian Ocean coast of South Africa. We quantified organochlorine pesticides, brominated flame retardants, and perfluorinated compounds (PFCs). Compared to 30 years ago, concentrations of ΣDDT have remained about the same or slightly lower, while ΣPCBs declined almost four-fold. The use of DDT in malaria control is unlikely to have contributed. PFCs were detected in all eggs. Indications (non-significant) of eggshell thinning associated with ΣDDT and ΣPCB was found. It seems therefore that the concentrations of measured organic pollutants the African Penguin eggs are not contributing directly to its current demise, but concerns remain about thinner shells and desiccation. Effects of combinations of compounds and newer compounds cannot be excluded, as well as more subtle effects on reproduction, development, and behaviour.

1. Introduction

The number of the African Penguins *Spheniscus demersus* (indigenous to South Africa and Namibia) declined from an estimated

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1.5–3 million early in the 20th century, to 179 000 in the early 1990s (Crawford et al., 2001), then to 52 000 birds in 2009 (Crawford et al., 2011, 2013). This precipitous decline resulted in this species being classified as Endangered (Crawford et al., 2011). The main reason for the decline is believed to be the reduction of the major fish prey species, the African sardine *Sardinops sagax* and the anchovy *Engraulis encrasicolus*. This decline in prey species abundance is suspected to be due to over-fishing, as well as oceanographic anomalies such as increased sea temperature and low levels of oxygen (Crawford et al., 2008a,b). The decline in fish stocks largely coincided with penguin mortalities, delayed onset of first breeding, and interrupted or depressed breeding success (Crawford et al., 2011). The recent eastward shift of sardine and anchovy pelagic shoals has also meant that penguins have to range further from breeding colonies in search of food, impacting on reproductive success (Crawford et al., 2011).

Another, possibly contributing cause to the population decline, namely the impact of chemical pollutants on reproduction, has not been investigated in more than 30 years, despite the globally recognised advantages of using seabirds to monitor chemical pollution in our marine systems (Mallory et al., 2010). Van Dyk et al. (1982) and de Kock and Randall (1984) were the only published literature that reported on concentrations of organic pollutants in African Penguin eggs (see Table 2). Since then, much more has become known about the potential and actual impacts of organic pollutants on seabirds. Concurrently, our knowledge of persistent organic pollutants (POPs) and other toxicants, such as brominated flame retardants (BFRs) and perfluorinated compounds (PFCs), that were not well known or investigated 30 years ago, came to the fore. The aim of this study was to determine the presence and concentrations of organic pollutants on the African Penguin, compare the results obtained to previous studies, and determine if the concentrations can be linked to the decline of this bird.

2. Materials and methods

2.1. Sample collection and sample preparation

The project was approved by the ethics committee of the North-West University (NWU) (NWU-00055-07-S3). Permits for collection of bird eggs were obtained from the relevant authorities. Fresh African Penguin eggs (10 eggs from each locality) were collected from two islands; Robben Island near Cape Town (Atlantic Ocean), and Bird Island near Port Elizabeth (Indian Ocean), South Africa (Fig. 1). Only one egg per clutch was collected. Van den Steen et al. (2011) found no significant differences in contaminant concentrations between 1st and 2nd eggs from Rockhopper Penguin *Eudyptes chrysocome* and Imperial Shag *Phalococorax atriceps* from the Falkland Islands and we assumed the same for this study. In addition, a single Kelp Gull *Larus dominicanus* and a single Cape Gannet *Morus capensis* egg were collected from Bird Island, and included here for reference and archival purposes. Eggs were wrapped in clean foil and frozen on the day of collection. Eggs were collected in late 2011 and early 2012. Both Cape Town and Port Elizabeth are cities with industrial, harbour, and residential activities, both therefore are potential sources of pollutants.

Sample preparation equipment was cleaned with soap and water, rinsed three times with double distilled water, and then washed three times with 96% ethanol. We analysed organochlorine pesticides (OCPs), including toxaphenes/chlorinated bornanes (CHBs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and perfluorinated compounds (PFCs) (Table 1). For the latter class we analysed PFHxS (sodiumperfuoro-1-hexasulphonate), PFDoA (perfluoro-n-dodecanoic acid), PFTrDA (perfluoro-tri-dodecanoic acid), PFOS (perfluoro-1-octane sulphonate), PFDA (perfluoro-n-decanoic acid), PFOA (perfluoro-n-octanoic acid) and PFNA (perfluoro-n-nonanoic acid). The eggs were thawed and the shells were carefully broken in the absence of direct light to prevent potential degradation of light-sensitive compounds. An ultrasonic homogeniser was used to homogenise the egg contents in such a way that as little foam as possible was formed. Samples were frozen and kept frozen during shipment to Norway. The samples were analysed at the Laboratory of Environmental Toxicology at the Norwegian University of Life Sciences in Oslo, which is an accredited laboratory (NS–EN ISO/IEC 17025 (TEST 137)) for testing biological materials of animal origins. The laboratory is accredited for all the compounds listed here, except for BDE-206, -207, -208 and -209, and the PFCs which are validated according to accreditation standard (NS–EN ISO/IEC 17025).

2.2. Extraction and analyses

The list of all compounds analysed is presented in Table 1. Procedures and quality control for extraction and analyses (gas-chromatography with electron capture (GC–ECD) and mass-spectrometry (GC–MS)) for the chlorinated and brominated compounds are according to Brevik (1978) and described and referenced in Bouwman et al. (2012) and Polder et al. (2008). Each sample series included one chicken egg blank, two recovery samples (homogenised chicken eggs, spiked with recovery standards), and three solvent blanks. The repeatability of the GC was confirmed by running a standard after every ten samples while reproducibility of the method was tested through analyses of the laboratory's in-house reference material (seal blubber). The DDT performance on the GC–ECD was continuously monitored. PFCs were analysed according Bytingsvik et al. (2012) and references therein by using liquid chromatography/negative electrospray ionisation tandem mass spectrometry (LC–ESI–MS/MS) gas-chromatography and mass spectrometry. The compounds detected, results of recoveries, and limits of quantification (LOQs) are listed in Table 1 as ng g^{-1}. LODs and are calculated as 3× noise level, except BDE-209 which were calculated as the mean of the blank value +2× standard deviations due to problems with blanks. Blank correction by subtraction was performed with procedural blanks. %DDT was calculated as the percentage of *p,p′*-DDT of the sum of all DDT compounds measured. Recoveries ranged between 88% and 127% for OCPs, 96–136% for toxaphenes, 96–114% for PBDEs, and 88–166% for PFCs. The data were not corrected for recoveries. We present and discuss the concentrations based on wet mass (wm) rather than lipid mass (lm). The reason is that embryonic metabolism does affect the lipid content of developing bird eggs (Romanoff, 1932) and because wet mass is also used as the basis for interpreting risk. For comparisons with other studies, we present summarised lipid-based concentrations (Tables 1 and 2).

2.3. Measuring eggshell thickness

The penguin eggshells were gently washed to remove the membrane, and allowed to dry for at least three weeks. The eggshell thickness was measured with an electronic digital calliper (0.01 mm). Three locations on the equator of the each shell was measured three times and the mean used for further calculations.

2.4. Data treatment

GraphPad Prism version 5.04 for Windows, GraphPad Software, San Diego California USA (www.graphpad.com) was used for descriptive analyses and comparisons using unpaired, two-tailed *t*-tests (Mann–Whitney nonparametric test comparing the distributions of two unmatched groups) on untransformed data that were normally distributed. Multivariate analysis of penguin egg