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From banana fields to the deep blue: Assessment of chlordecone contamination of oceanic cetaceans in the eastern Caribbean

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ABSTRACT

In the French West Indies (Caribbean), the insecticide Chlordecone (CLD) has been extensively used to reduce banana weevil (*Cosmopolites sordidus*) infestations in banana plantations. Previous studies have shown high CLD concentrations in freshwater and coastal communities of the region. CLD concentrations, however, have not yet been assessed in marine top predators. We investigated CLD concentrations in cetacean blubber tissues from Guadeloupe, including *Physeter macrocephalus*, *Lagenodelphis hosei*, *Stenella attenuata* and *Pseudorca crassidens*. Chlordecone was detected in all blubber samples analysed, with the exception of four *P. macrocephalus*. Concentrations (range: 1 to 329 $ng \cdot g^{-1}$ of lipid weight) were, however, lower than those found in species from fresh and brackish water. Ecological factors (open ocean habitat), CLD kinetics, and cetacean metabolism (high or specific enzymatic activity) might explain low concentrations found in cetacean blubber. Future analyses that include internal organ sampling would help to confirm CLD levels observed in this study.

1. Introduction

Chlordecone (also known as Kepone, CLD) is an organochlorine insecticide once used worldwide (Europe, USA, Latin America, Africa and Asia) to control banana weevil (*Cosmopolites sordidus*) infestations in banana plantations, including in the French West Indies (FWI) (Fintz, 2009; Le Déault and Procaccia, 2009). This molecule is highly persistent in the environment (Cabidoche et al., 2009), and biomagnifies through food webs (UNEP, 2006; Coat et al., 2011; Dromard et al., 2018). Therefore, this compound, which poses a significant risk for wildlife and human populations (Cabidoche et al., 2009; Coat et al., 2011; Multigner et al., 2010) is still being found in the local environment (i.e. soils, rivers, spring water, etc.) despite having been banned since 1993 in the FWI. Chlordecone can induce a wide range of pathologies in birds and mammals, including reproductive impairment or neurotoxicity (Epstein, 1978; Huff and Gerstner, 1978). It is carcinogenic and has been shown to cause hepatic tumours in laboratory rats and mice (Sirica et al., 1989), but also prostate cancer in humans (Multigner et al., 2010). The carcinogenic and hormonal properties of CLD and its long biological half-life raise the possibility of long-term effects. For all these reasons, CLD was prohibited by the Stockholm Convention in 2009 (UNEP, 2017).

The first assessment of CLD contamination in the FWI was conducted in soil and aquatic organisms from the rivers of Guadeloupe in late 70's (Snegaroff, 1977; Kermarrec, 1980), when it was still in use. Bocquené (2002) and Bocquené and Franco (2005) highlighted CLD contamination after the ban in the suspended organic matter and sediments in rivers of Martinique (FWI), and for the first time, CLD contamination in two marine species (*Acanthurus bahianus* and *Panulirus argus*). More recently, studies have expanded to a diversity of taxa from coastal ecosystems, and on the ecological drivers of observed concentrations such as foraging habitat preferences (e.g. Coat et al., 2006; Bodiguel et al., 2011; Salvat et al., 2012; Dromard et al., 2016; Dyc et al., 2015).

CLD is highly lipophilic with a log Kow (octanol-water partition coefficient) between 4.5 and 6.0 (Howard et al., 1991; Hansch et al., 1995). Consequently, CLD tended to be associated to organic particulate matter and is prone to biomagnification and bioaccumulation in food webs (UNEP, 2006). However, little information has been reported in marine wildlife, and no information was available on CLD

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concentrations in marine top predators such as marine mammals in the Eastern Caribbean. Therefore, the aim of this study is to provide the first information of CLD contamination in the pelagic marine environment analysing the blubber of four species of odontocete cetaceans from the west coast of Guadeloupe Island, FWI.

2. Material and methods

2.1. Sample collection and species studied

Samples were collected off the leeward coast of Guadeloupe (16° 15′ N, 61° 34′ W), in the FWI in April 2015. Skin and blubber biopsy samples of cetaceans were obtained opportunistically during boat-based cetacean surveys. When groups were encountered, individual animals were sampled using a crossbow (BARNETT Veloci-Speed® Class, 68-kg draw weight) with Finn Larsen (Ceta-Dart, Copenhagen, Denmark) bolts and tips (dart 25-mm long, 5-mm-diameter). The animals were hit below the dorsal fin when sufficiently close (5–15 m) to the research boat and samples were preserved individually frozen at −20 °C before shipping and subsequent analysis. CLD analyses were performed using the blubber.

A total of 46 individuals belonging to four cetacean species having different feeding habits and habitats were sampled: sperm whales (*Physeter macrocephalus*, $n = 10$) are resident population in the Eastern Caribbean that mainly fed on mesopelagic cephalopods (Whitehead, 2003; Gero et al., 2014), false killer whales (*Pseudorca crassidens*, $n = 1$) occur in deep oceanic and insular slope waters of tropical archipelagos and mostly feed on high trophic level epipelagic fish (Würsig et al., 2018). Fraser's dolphins (*Lagenodelphis hosei*, $n = 5$) also occur in deep oceanic waters, but feed on lower trophic level mesopelagic fishes (myctophids), cephalopods, and crustaceans (Dolar et al., 2003; Wang et al., 2012). The pantropical spotted dolphin (*Stenella attenuata*, $n = 30$) has a relatively similar distribution and foraging behaviour than Fraser's dolphins, but also feed on epipelagic prey (Wang et al., 2012).

Biopsy sampling was conducted under scientific permit delivered by DEAL Guadeloupe (12 February 2015, Autorisation Préfectorale de Dérogation pour la Perturbation Intentionnelle de Spécimens d'Espèces Animales Protégées).

2.2. Analyses of chlordecone (CLD) concentrations

Blubber tissue from biopsy samples was used for CLD analysis due to the lipophilic nature of this molecule. Blubber was cut and ground and anhydrous (Na_2SO_4) and surrogates (i.e. PCB 103 and 198) were added to the samples as well as to the blanks and reference material prior to extraction. The samples were extracted with 80 mL of dichloromethane using a Soxhlet apparatus for 8 h. The extract was concentrated to 2 mL by rotary evaporation, of which 200 µL were used to determine the amount of lipids through gravimetry. The remaining extract was cleaned with 4 mL of sulphuric acid (H_2SO_4, 95–97%) in order to remove organic matter (lipids, lipoproteins, carbohydrates). The organic phases were collected into a new tube and the hexanic layer was extracted with another volume of 2 mL of distilled water to eliminate residues of H_2SO_4. Then, that phase was further cleaned up with 3.2 g of solid Florisil (magnesium-silicate) adsorption column and target compounds were eluted with a mixture of *n*-hexane/dichloromethane (1:1, V:V). The eluate was then concentrated with a gentle flow of nitrogen to 0.9 mL. Prior to gas chromatographic analysis, an internal standard [2,4,5,6 tetrachloro-*m*-xylene (TCMX)] was added. CLD was quantitatively analysed through an Agilent Technologies 7890B gas chromatograph with tandem mass spectrometer (GC/MS/MS) 7010B using an ultra-inert capillary column coated with 5% phenyl-substituted dimethylpolysiloxane phase (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The volume injected was 1 µL in automatic splitless mode. Helio was used as the carrier gas (constant flow of 1.1 mL·min^{-1}). The interface, source and quadrupole temperatures were 280 °C, 300 °C and 200 °C, respectively. Oven temperature was programmed as follows: 75 °C for 3 min, raised at 15 °C min^{-1} to 150 °C, then raised at 2.0 °C min^{-1} to 260 °C and at 20 °C min^{-1} to 300 °C with a final hold of 10 min. The multiple reactions monitoring (MRM) used for CLD was 272–237 and 272–235, as the qualifier ion, using collision energy of 20 eV. Analytical curves were generated from eight different concentrations of reference standard purchased from Sigma-Aldrich.

The methods used were validated by the replicate analysis of standards and samples as well as through spiking (i.e. addition of known concentrations of all analytes before analyses). The analysis of standard reference material (SRM1945; organics in whale blubber from National Institute of Standards and Technology - NIST) was carried although there is no certified or reference concentration for CLD for comparison. Surrogate recovery ranged from 80% to 120% and spiked matrices were recovered within the acceptable ranges (i.e. 50 to 120% for at least 80% of spiked analytes, as suggested by Wade and Cantillo, 1994). All concentrations were blank subtracted and expressed in ng·g^{-1} of wet and lipid weight to facilitate comparisons with other matrices.

2.3. Sex determination

The sex of each sample was determined using left over skin samples and PCR analysis. DNA was extracted from a small piece of skin using the pheno-chloroform method. PCR analysis was performed by simultaneously targeting the ZFX gene (forward primer 5′-ATAGGTCTG CAGACTCTTCTA-3′, reverse primer 5′-AGAATATGGCGACTTAGA ACG-3′; Bérubé and Palsbøll, 1996) and SRY gene (forward primer 5′-CATTGTGTGGTCTCGTGATC-3′, reverse primer 5′-ACCGGCTTTCCATT CGTGAACG-3′; Rosel, 2003). Briefly, PCR reactions were conducted using Quanta© PCR kits. The final reaction volume for each sample was 25.1 µL consisting of 2.5 µL PCR Buffer, 1 µL of each Primer (ZFX Forward and Reverse and SRY Forward and Reverse), 1 µL $MgSO_4$, 0.5 µL dNTPs, 15 µL water, 0.1 µL Taq Polymerase, and 2 µL of DNA (concentration 10 ng·µL^{-1}). The following thermocycler profile was used: 92 °C for 30s followed by 35 cycles of 94 °C for 30s, 51 °C for 45 s, 68 °C for 45 s, and then ending with 68 °C for 1 min with 4 °C hold. Fragment patterns were visualized on a 2.5% agarose gel, with males having a band for both ZFX and SRY genes and females having one band for the ZFX gene.

2.4. Data analysis

Differences in chlordecone concentrations among species were tested using the non-parametric Kruskal-Wallis test, followed by Dunn-Bonferroni post hoc test. *P. crassidens* has been removed from this analysis since only one sample was available. To infer concentrations difference between sexes of *Stenella attenuata* the also non-parametric Wilcoxon-Mann-Whitney test was used. For the other three species, the effect of sex on CLD concentrations was not tested since only females were sampled.

The level of significance for statistical analyses was set at $\alpha = 0.05$ and analyses were performed using Rstudio Team version 1.0.136 (RStudio Team, 2016).

3. Results and discussion

Chlordecone was present in all the blubber samples analysed with the exception of four *P. macrocephalus* (Pm_6, 7, 8 and 10) (Table 1). Only *P. macrocephalus* and *S. attenuata* showed significant differences on CLD concentrations (Kruskal-Wallis post-hoc test, $p = 0.0067$), moreover there is no significant difference among sexes of this last species (Wilcoxon, $p > 0.05$).

There was, however, considerable variation among individual sperm whales in recorded CLD concentrations (LQ – 34.9 ng·g^{-1} ww) and *S. attenuata* had the highest median values, followed by *P.*