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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.*

Table 1
Blubber concentrations of chlordecone (CLD) expressed in ng·g^{-1} of wet and lipid weight (ww and lw, respectively), lipid content (%) and sex of all the individuals analysed for the four species. Pm = *Physeter macrocephalus*, Lh = *Lagenodelphis hosei*, Sa = *Stenella attenuata*, Pc = *Pseudorca crassidens*. M = male, F = female.

ID	Species	Sex	Lipid	CLD ww	CLD lw
Pm_01	Pm	F	20.0	34.9	175
Pm_02	Pm	F	10.4	0.787	7.58
Pm_03	Pm	F	0.4	1.35	329
Pm_04	Pm	F	0.9	1.73	198
Pm_05	Pm	F	11.0	0.544	4.96
Pm_06	Pm	F	7.1	< LQ	< LQ
Pm_07	Pm	F	9.0	< LQ	< LQ
Pm_08	Pm	F	1.1	< LQ	< LQ
Pm_09	Pm	F	3.5	0.206	5.89
Pm_10	Pm	NA	7.3	< LQ	< LQ
Lh_01	Lh	F	21.3	1.95	9.14
Lh_02	Lh	F	10.5	1.03	9.76
Lh_03	Lh	F	14.2	1.91	13.5
Lh_04	Lh	F	6.7	0.679	10.1
Lh_05	Lh	F	8.5	6.73	79.1
Sa_01	Sa	F	20.5	9.05	44.3
Sa_02	Sa	F	11.4	6.79	59.4
Sa_03	Sa	M	27.8	6.95	24.9
Sa_04	Sa	F	14.8	3.60	24.3
Sa_05	Sa	M	11.9	1.26	10.6
Sa_06	Sa	F	12.6	3.09	24.6
Sa_07	Sa	M	33.4	5.63	16.8
Sa_08	Sa	M	22.1	3.95	17.9
Sa_09	Sa	M	20.4	4.00	19.6
Sa_10	Sa	F	27.7	4.86	17.6
Sa_11	Sa	M	14.4	2.07	14.3
Sa_12	Sa	M	10.4	12.3	119
Sa_13	Sa	M	20.6	3.70	18.0
Sa_14	Sa	F	2.4	1.92	81.0
Sa_15	Sa	F	21.4	9.35	43.8
Sa_17	Sa	F	16.8	6.17	36.7
Sa_18	Sa	M	29.5	4.14	14.0
Sa_19	Sa	F	14.0	6.15	43.9
Sa_20	Sa	F	19.5	5.45	28.0
Sa_21	Sa	F	24.8	5.54	22.4
Sa_22	Sa	F	13.6	4.02	29.5
Sa_23	Sa	M	17.7	4.46	25.2
Sa_24	Sa	F	29.0	9.70	33.5
Sa_25	Sa	F	20.9	4.51	21.6
Sa_26	Sa	M	29.6	3.72	12.6
Sa_27	Sa	M	28.2	6.94	24.6
Sa_28	Sa	M	20.5	3.33	16.3
Sa_29	Sa	M	19.3	5.82	30.2
Sa_30	Sa	M	28.9	4.62	16.0
Pc_01	Pc	M	34.5	3.92	11.4

NA = Not available.
LQ = Limit of quantification (= 1 ng·g^{-1} lw).

crassidens, *L. hosei* and *P. macrocephalus* (4.62 > 3.92 > 1.91 > 0.374 ng·g^{-1} ww, Fig. 1). These concentrations are lower than those found by Coat et al. (2011) in other marine taxa (e.g. American eel, *Anguilla rostrata*, 5863 ng·g^{-1} ww) and considerably lower compared to fresh and brackish water species (e.g. river goby, *Awaous banana*, 1350–12,366 ng·g^{-1} ww and wild tilapia, *Oreochromis* spp., 196–386 ng·g^{-1} ww; Coat et al., 2006, 2011). CLD concentrations in cetaceans were lower compared to those reported in two sea turtle species from Guadeloupe, which ranged from the limit of quantification to 378 for the green turtle (*Chelonia mydas*) and to 26.7 ng·g^{-1} ww for the hawksbill turtle (*Eretmochelys imbricata*) (Dyc et al., 2015).

The use of CLD was banned in 1990 in France, but used by exemption until 1993 in the FWI. Nevertheless, this molecule is still detected in the environment and high concentrations were found in terrestrial products (meat, milk and eggs), root vegetables (DIREN, 2001; DSDS, 2001; Cabidoche et al., 2009), as well as in fresh and marine

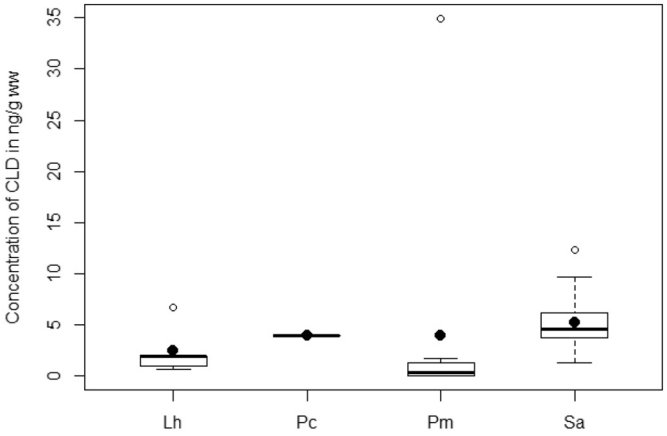

Fig. 1. Median and mean concentrations of chlordecone (CLD) concentrations (ng·g^{-1} wet weight, ww) in the four species studied. Mean concentrations are represented as black dots. Lh = *Lagenodelphis hosei*, Pc = *Pseudorca crassidens*, Pm = *Physeter macrocephalus* and Sa = *Stenella attenuata*.

water ecosystems in the FWI after the ban (e.g. fish, crustaceans and marine turtles; Coat et al., 2006, 2011; Dyc et al., 2015; Dromard et al., 2016). Coat et al. (2011) investigated CLD concentrations in a large number of aquatic animal species (fresh and marine ecosystems) collected in 2006 in Guadeloupe. This previous study revealed a significant positive relationship between CLD concentrations and the trophic position of sampled species supporting the biomagnification of this pollutant along food webs. They also observed that species from the same genus within a trophic level exhibited contamination levels twice as high when living in static waters (e.g. *Macrobrachium faustinum* and *M. acanthurus*) than in rapid running waters (e.g. *M. heterochirus* and *M. crenulatum*) (Coat et al., 2011). In addition, CLD concentrations were also correlated with foraging habitat preferences (carbon sources) inferred from $\delta^{13}C$ values. Indeed, three of the four fish species studied (*Anguilla rostrate*, *Eleotris perniger*, *Awaous banana*) that exhibited the highest CLD concentrations fed on ^{13}C-enriched food sources (Coat et al., 2011). Therefore, considering that cetaceans are high trophic level consumers in marine ecosystems we were expecting relatively higher CLD concentrations in blubber tissues than those we observed.

Our finding of relatively low CLD concentrations could be due to several non-mutually exclusive factor including 1) the ecology and biology of the species investigated (i.e. offshore foraging habitats spatially removed from pollution sources, relative trophic level) 2) CLD kinetics and its special affinity for particular tissues or organs of the body (i.e. organotropism) not analysed here and 3) the species physiology concerning its ability to metabolise the molecule.

CLD concentrations in zooplankton communities were higher in samples collected at a river mouth than those collected in more marine habitats, suggesting chemical dilution of CLD in coastal ecosystems (Coat et al., 2011; Dromard et al., 2017). The four species investigated were sampled in oceanic waters (depth > 1000 m) and their food webs are spatially removed from terrestrial and freshwater sources of CLD pollution. In the tropical Atlantic, *S. attenuata* is an epipelagic predator occurring in slope and oceanic waters (Moreno et al., 2005), and feed on epi- and mesopelagic prey (Perrin, 2018). CLD concentrations were the highest for this species (Fig. 1). *P. macrocephalus* occurs in deep oceanic waters and feeds on mesopelagic prey (Whitehead, 2003). Therefore, it is not surprising that we found generally low concentrations of CLD in samples. Less clear is why there was such a range of individual CLD levels detected (see Table 1). Sperm whale groups, however, range widely and may encounter different conditions or levels of coupling of nearshore and offshore food webs. Further investigations that account for differences in age (bioaccumulation), social and spatial dynamics (resident vs transient individuals), and including males in sampling, are needed.

Blubber was the tissue used to CLD analysis. This matrix has been used for monitoring organochlorine compounds levels in marine mammals (Wagemann and Muir, 1984) since it is lipophilic, highly apolar and dissolve in neutral lipids. Therefore, POPs highly concentrate in the blubber of marine mammals. However, the distribution of CLD among organs in an individual (organotropism) is essential information that should be considered when interpreting the low concentrations we found in blubber. Toxicodynamics and toxicokinetics determine a molecule's behaviour in an organism and especially its distribution, through parameters such as its structure, implying affinity for tissues, its absorption, bioavailability, transformation controlled by enzymes and elimination rates. Moreover, the lipophilic properties of CLD can also drive variation in the distribution of the molecule among tissues and organs. This propriety also affects the pollutant retention time within an animal organ as a function of body fatness. Studies in a variety of taxa suggest that CLD is mainly concentrated in liver and muscle rather than in fat (e.g. humans, growing goats, ducks, other farm animals; Bouveret et al., 2013; Jondreville et al., 2014a, 2014b; Jurjanz et al., 2014; Lastel et al., 2016). Several studies suggested that CLD distribution depends on its affinity for blood components. In fact, CLD has a high affinity to albumin and high-density lipoprotein (HDL) (Skalsky et al., 1979; Guzelian, 1982; Soine et al., 1983), and liver has many receptors for albumin. Therefore, the links CLD-albumin and CLD-HDL appear to explain the high concentration of this pollutant found in the liver of several different farm species as well as in humans (e.g. Lastel et al., 2016). Nevertheless, CLD distribution in adipose tissues, like blubber, is more related to the development of these tissues within the organism and to the different lipid classes which compose adipose tissues. Then, the HDL, for which CLD has a high affinity, are richer in cholesterol and phospholipids than in triglycerides (Hocquette and Bauchart, 1999). In that sense, lipid class analyses in blubber have indicated that this tissue is almost entirely composed of triglycerides (Olsen and Henderson, 1989).

Other contributing factors that should be considered come under the CLD kinetics, especially the half-life of the molecule, and its transformation in organisms. Studies conducted in ruminants determine that CLD has a low half-life in comparison with other organic pollutants (e.g. 12–22 days for growing piglets, 15 days for growing juvenile goats, 20 days in cow milk or 43 days in the muscle of beef), which depends on the species but not depend on the levels of exposure (Soine et al., 1983; Lastel et al., 2016; Smith and Arant, 1967; Mahieu et al., 2015; Fournier et al., 2017). Moreover, CLD can be converted to various metabolites and particularly to chlordecol (CLD-OH) through the intervention of an enzyme: the aldo-keto reductase (Fariss et al., 1980). This enzyme is not present in all species, or its level of activity is species dependant, as highlighted for gerbil that showed higher activity than in rat or guinea pig (Houston et al., 1981).

In conclusion, the presence of chlordecone in the blubber of cetaceans revealed that this controversial and persistent molecule has reached deep sea food webs in areas with deep waters close to shore. However, the low concentrations we found compared to other aquatic organisms likely are the result of dilution of the molecule with distance from points of origin and the ecology of the species we studied (i.e. found in open ocean habitats and food webs), CLD kinetics and cetacean metabolism (high enzymatic activity). Around tropical islands, stranded cetacean carcasses are quite infrequent and rarely accessible for internal organ tissue collection. Nevertheless, using existing stranding networks in the French West Indies, future studies using internal organ sampling will be conducted to compare CLD concentrations between organs in order to confirm levels reported in this study.

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