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Concentrations of DDE in Blubber Biopsies of Free-Ranging Long-Beaked Common Dolphin (*Delphinus capensis*) in the Gulf of California

Juan Pablo Gallo-Reynoso · Tiffany B. Malek · Jaqueline García-Hernández · Luz Vázquez-Moreno · Iris Segura-García

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Abstract Long-beaked common dolphins (*Delphinus capensis*) in the Gulf of California have been exposed to persistent contaminants that originated in large agricultural areas near the coast. Live common dolphins were sampled by remote dart biopsies to determine concentrations of tDDT in blubber. Life stage and initial gender identification was determined by field observations. Gender was confirmed by genetic analysis of the skin. Concentration of tDDT in blubber was analyzed by gas chromatography. The 16 samples collected consisted of: 2 adult males, 6 adult females, and 8 juveniles. 4,4′-DDE was detected in most of the samples with 4,4′-DDD and 4,4′-DDT under detection levels. Concentrations of DDE varied from non-detectable to 87.3 µg/g lipid weight with a median of 16 µg/g lipid weight. The highest concentration was detected in an immature female. No differences were detected between gender or life stage but this could be attributed to small sample size. We recommend continued sampling of *D. capensis* blubber biopsies from the Gulf of California in order to relate these levels with affected in vitro biomarkers such as mixed function oxidase activity.

Keywords 4,4′-DDT · Common Dolphins · *Delphinus capensis* · Gulf of California

Delphinus capensis is a coastal-pelagic species with schools moving seasonally along the Gulf of California: aggregating towards the northern half of the gulf in spring–summer with migration to the southern half of the Gulf in autumn–winter. Long-beaked common dolphins gather in schools ranging in size from 50 to 5,000 individuals with occasional aggregations of more than 10,000 individuals (Gallo-Reynoso 1991). Schools form mainly to feed on fish with high fat content such as Monterey sardine (*Sardinops caerulea*), threadfin herring (*Opisthonema* spp.), Japanese sardine (*Etrumeus teres*), anchovy (*Cetengraulis mysticetus*), Pacific mackerel (*Scomber japonicus*), lantern fishes (*Triphoturus mexicanus* and other myctophids), and neritic squids (*Lolliguncula panamensis*, *Loliolopsis diomedae*) (Gallo-Reynoso 1991; Niño-Torres et al. 2006; Gallo-Reynoso unpublished data).

Agricultural run-off into the Gulf of California occurs from the Mexicali, Yaqui, and Culiacan agricultural valleys in Baja California, Sonora, and Sinaloa, where DDT was intensively used in the 1960-1970s (SAGAR 1972). However, few studies have reported concentrations of DDT in marine mammals from this gulf (Calambokidis 1988; Niño-Torres et al. 2009). Long-beaked common dolphins are reliable indicators of organochlorine compounds present in the Gulf of California due to their limited distribution, higher position in the food chain and high fat diet. DDT is a lipophilic xenobiotic that is known to concentrate in fatty stores of marine mammals at high levels (Gaskin 1982). Concentrations in blubber have been associated with

J. P. Gallo-Reynoso (✉) · J. García-Hernández
Centro de Investigación en Alimentación y Desarrollo A. C., Carretera a Varadero Nacional, Km. 6.6, Colonia Las Playitas, C.P. 85480 Guaymas, Sonora, Mexico
e-mail: jpgallo@ciad.mx

T. B. Malek
Illumina, Inc., 5200 Illumina Way, San Diego, CA 92122, USA

L. Vázquez-Moreno
Centro de Investigación en Alimentación y Desarrollo A. C., Carretera a la Victoria, Km. 0.6, C.P. 83000 Hermosillo, Sonora, Mexico

I. Segura-García
Durham University, Old Elvet, Durham DH1 3HP, UK

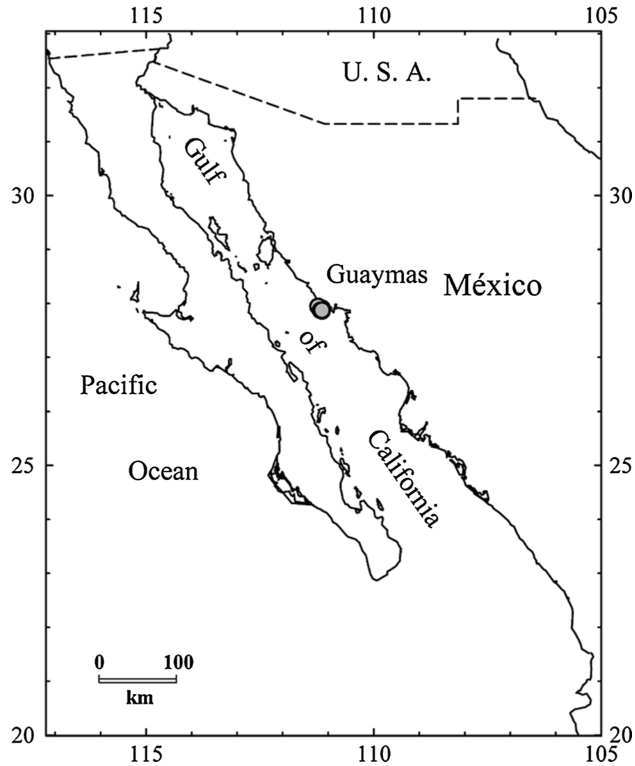

Fig. 1 Location (*gray circles*) where remote dart biopsies of *Delphinus capensis* were collected in 1999 in the Gulf of California

reproductive impairment and suppressed immune systems in marine mammals, both detrimental to the long-term health of a population (Borrell et al. 1995). Here we present results of DDT metabolite concentrations in 16 blubber biopsies of free-ranging long-beaked common dolphins collected off the coast of Guaymas, Sonora, Mexico.

Methods

Sample Collection

Samples were collected from May 3 to May 20, 1999, 10 miles offshore Guaymas Sonora, Mexico (27°51′51N–27°56′58N and 111°08′03W–111°13′51W; Fig. 1). Depths in this area range from 260 to 330 m. Blubber samples were collected by remote dart biopsies shot from the pulpit of a 24-foot long fiberglass skiff. Upon sighting, dolphin schools were approached to a shooting range of 2–10 m (Focardi et al. 1991). Sampling dart construction consisted of a light aluminum bolt with a modified stainless steel collecting tip and floater. Darts were fired with a Barnett Wildcat II crossbow (Tarpon Springs, FL). To prevent possible infection, bolt tips were sterilized through submersion into 95 % alcohol between shootings. Biopsies were taken from a circular area of approximately 40 cm in diameter near the dorsal fin, in the flank area above the flippers. Each biopsy resulted in a cylindrical-shaped core of skin and blubber sample of 1.2 cm in length. Dolphin reactions to biopsy darts were an immediate dive and a hard tail flick followed by high-speed swimming from the bow of the boat (reaction nomenclature from Weinrich et al. 1992).

Dolphin Identification

Adult males were recognized by the presence of a ventral keel, dark dorsal coloration, and large size (Gallo-Reynoso 1991; Vidal and Gallo-Reynoso 2012). Juveniles and subadult individuals of undetermined sex were also sampled. Sex of each individual sample was confirmed with DNA analysis of the skin. Biopsy samples were taken from individuals of both sexes. A total of 16 blubber biopsies were collected from a variety of age and gender groups.

Lipid Extraction and Analysis

Sterile forceps were used to remove samples from the bolt tip, placed in a vial and stored on ice at 4°C for 2–3 h. At the laboratory, dolphin skin was excised from the sample with a sterile scalpel, placed in 95 % ethanol and stored in a freezer at −20°C for genetic analysis. The blubber was placed in a vial and stored at −20°C until extraction. Biological duplicates were created from those samples with at least two grams of lipid present. Samples were extracted following the Folch method (Folch et al. 1957). One gram of lipid tissue was homogenized with chloroform/methanol (2:1) to a final volume of 20 mL. After dispersion, the homogenized mix was agitated for 15–20 min in an orbital shaker at room temperature. The homogenate was then filtered to recover the liquid phase. The solvent was washed with 4 mL of water, vortexed for a few seconds, and centrifuged at low speed (2,000 rpm) to separate the two phases. The upper phase was removed by siphoning and the lower chloroform phase, containing the lipids, was evaporated with a vacuum under a nitrogen stream. The sample extract was brought to a volume of 100 mL. Percent lipid content was determined gravimetrically by drying a 5 mL aliquot of the sample extract. Remaining sample extract was sent to a certified (Mexican Accreditation Entity or EMA) Toxic Residues laboratory at the Centro de Investigación en Alimentación y Desarrollo (CIAD) in Hermosillo, Sonora. Samples were processed according to the official regulation NOM-021-ZOO-1995 for the analysis of organochlorine pesticides and PCBs in equine, bovine, porcine and poultry blubber by gas chromatography, published in the official gazette (Diario Oficial de la Federación 1995). Lipid samples were mixed with alumina and seeded in a column with alumina as stationary phase and *n*-hexane as the eluent. The eluate was concentrated by evaporation and further analyzed in a gas chromatograph with an electron capture detector (ECD). For quality control/quality assurance (QC/QA) purposes, two blank fat tissue samples were fortified during extraction with certified Ultra Scientific® (Kingstown, RI) 99 % purity solutions of 4,4′-DDE, 4,4′-DDD and 4,4′-DDT. In addition of fortified samples, each batch of extraction samples included a baseline solution, glassware blank, solution blank and a fat tissue blank. Percent recovery of fortified samples were 93 %–98 % for 4,4′-DDE, 96 %–103 % for 4,4′-DDD and 94 %–98 % for 4,4′-DDT. Detection limits for 4,4′-DDE and 4,4′-DDD was 0.053 μg/g and for 4,4′-DDT it was 0.267 μg/g (lipid weight), concentrations lower than these values were considered as <ND (under detection limit). All analyzed blanks were <ND. Results are expressed in relation to the total lipid content of the tissue sample.

Results and Discussion

A total of 16 common dolphins were biopsied: 2 adult males, 6 adult females, 3 juvenile males and 5 juvenile females. Percent coefficient of variation in duplicates averaged 19, with only three samples exceeding 24 percent. 4,4′-DDE was the only compound detected in most of the samples; 4,4′-DDD and 4,4′-DDT were analyzed but not detected. Concentrations of 4,4′-DDE varied from non-detectable (<DL) to 87.3 μg/g lipid wt, with a median of 16 μg/g lipid wt (quartiles 10.0–22.0 μg/g lipid wt). The highest concentration of 4,4′-DDE was present in an immature female (Table 1). Due to the small sample size, comparisons between males and females or between adult and juvenile organisms could not be made. A sample size of at least 70 males and 70 females would provide more power to detect a difference in 4,4′-DDE content, with 90 % power and an alpha of 0.05.

Table 1 Concentration (range) of 4,4′-DDE (μg/g lipid wt) and lipid content (range, %), in blubber samples of male (M), female (F) and juvenile (J) common dolphins (*Delphinus capensis*) from the Gulf of California, Mexico collected in 1999

Sample ID	Sex	Lipids (%)	4,4′-DDE
DC070599-1	M	19.1–21.4	18.0–23.7
DC070599-3	M	4.9	10.0
Median		19.1	18.0
Geom. mean		15.1	17.2
DC110599-5	F	22.2–23.2	11.3–15.2
DC180599-2	F	13.3–16.2	49.3–51.0
DC180599-3	F	12.5–14.1	4.8–21.3
DC180599-5	F	25.3–26.6	16.0–16.4
DC110599-2	F	24.0	12.9
DC180599-7	F	7.5	21.5
Median		19.2	16.2
Geom. mean		18.5	22.0
DC070599-2	M–J	19.1–23.6	11.5–16.2
DC180599-1	M–J	17.4–19.2	5.8–14.8
DC110599-4	M–J	1.0[a]	<DL
Median		19.1	11.5
Geom. mean		16.0	12.1
DC070599-4	F–J	17.4–19.7	17.3–21.3
DC110599-1	F–J	30.6–39.5	4.9–10.0
DC180599-4	F–J	23.6–29.5	25.2–28.3
DC180599-6	F–J	8.3–30.0	<DL
DC110599-6	F–J	4.1	87.3
Median		23.6	17.3
Geom. mean		22.5	21.5

[a] Lipid (%) in this dolphin was low because the biopsy dart struck the back of the dolphin in a very acute angle, obtaining more skin than blubber. This DDE concentration was excluded from the statistical analysis

Most reports of DDT concentrations in marine mammals have been obtained from stranded animals (Kuehl et al. 1991; Salata et al. 1995; Stokin et al. 2007). This type of collection is valuable as sample size can be quite large due to mass mortalities (Kuehl et al. 1991). However, stranded animals often die as consequence of disease, and these animals may carry abnormal pollutant loads (Aguilar et al. 1999; Hansen et al. 2004). Live animals are more accurate indicators of contaminant exposure in a population. Our results are therefore indicative of concentrations found in the free-ranging population of common dolphins in the Gulf of California.

In 1972, approximately 240,000 kg of the active ingredient DDT was applied to cotton crops in the Mexicali valley (SAGAR 1972). More than 30 years later, DDT residues are still present in fish from the Colorado River delta with concentrations from 5 to 340 ng/g (Garcia-Hernandez et al. 2001). Therefore, the intensive agriculture use of DDT in the past is the likely source of DDE residues in the common dolphins. Concentrations of tDDT in male common dolphins from the Gulf of California can be considered as medium range values; however, concentrations in females were higher than the majority of what has been reported in other studies (Table 2).

When compared to studies of temperate zones such as the North Atlantic, the ratio of DDE/tDDT detected in this study is high (Table 2). Common ratios of DDE/tDDT for temperate zones are leveled at approximately 60 % in pinnipeds and odontocetes (Aguilar 1984). However, in marine mammals of the Gulf of California, ratios appear to be higher. For example, the DDE/tDDT ratio for the vaquita (*Phocoena sinus*), an endangered species from the upper Gulf of California, was 93 % (Calambokidis 1988).

Table 2 Concentration of tDDT (μg/g lipid wt) ordered from oldest to recent record and % 4,4′-DDE/tDDT ratio, in common dolphins from around the world including our study from the Gulf of California

Species[a]/area (references)	Years	N	% Lipid	tDDT geometric mean (μg/g)			% (4,4′-DDE/ tDDT)			Coll.[b]	Wt.[c]
				Males	Fem.	Juv.	Males	Fem.	Juv.		
DD/Atlantic coast-Spain (Aguilar 1984)	1979	1		61.33			60			U	L
DD/Atlantic coast-US (Kuehl et al. 1991)	1986–1988	4		14.43						S	L
DD/South of Ireland (Smyth et al. 2000)	1990–1994	8	83.0	9.44	3.99		81	59		N	L
DD/Southern Mediterranean (Borrell and Aguilar 2005)	1992–1994	26	66.0	157.00	3.00	31.00	80	70	49	N	L
DD/Southwestern UK (Law et al. 2013)	1992–1998	20	85.0		4.66					S	L
DD/Southern Mediterranean (Fossi et al. 2003)	1994–1998	13		7.00						B	F
DD/Atlantic Ocean (Borrell et al. 2001)	1996	54	51.5	9.51	5.12		62	52		N	L
DC/Brazilian coasts (Kajiwara et al. 2004)	1997–1999	1	57.0	11.00						N	L
DC/Gulf of California (this study)	1999	16	20.1	15.43	21.18	20.62	>97[d]	>98	>98	B	L
DD/Southwestern UK (Law et al. 2013)	1999–2006	23	81.5		2.20					S	L
DC/Brazilian coasts (Lailson-Brito et al. 2012)	2000–2005	4	41.0	38.45	0.45					N	L
DD/Atlantic coast-Spain (Tornero et al. 2006)	2001–2003	74	64.1	6.27	2.07	3.52				N	L
DD/Adriatic sea (Lazar et al. 2012)	2004	1	14.0	105.90			66			S	L

[a] Species: DD, *Delphinus delphis*; DC, *Delphinus capensis*

[b] Collection method: U, unknown; S, stranded; N, netted; B, blubber biopsies

[c] Analytical normalization: L, Lipid wt; F, fresh wt

[d] Approximation of ratios calculated using detection limit of 4,4′-DDD and 4,4′-DDT

Sea lions from the central and southern Gulf of California, have reported ratios between 88 % and 96 % (Niño-Torres et al. 2009). Higher mean ratios (% DDE/tDDT) have been reported in male bottlenose dolphins from south India (93 %) (Tanabe et al. 1993) and the Florida coast (92 %; Hansen et al. 2004). It is possible, that the rate of dehydrochlorination of DDT to DDE in the environment could be accelerated in warmer waters. Laboratory experiments that mimic climatic conditions in India, observed an increase in DDE percentage from 11.6 % at 15°C to 40 % at 30°C in flooded soils (Samuel and Pillai 1989). Temperatures in the Gulf of California during summer average 26°C with variations between 15 and 32°C in the central Gulf of California, these temperatures could be even higher at the coast, which may result in higher rates of dehydrochlorination of DDT in coastal sediments. Higher concentrations of DDE also indicate no new inputs of DDT. High values of the DDE/tDDT ratio have been frequently associated with old inputs of pollution in the ecosystem (Aguilar 1984).

Transfer of persistent chemicals occurs by consuming low trophic level aquatic biota as food by higher trophic level biota through the process of biomagnification (Connell 1998). In the case of common dolphins, biomagnification of DDT is given by the consumption of low trophic organisms such as sardines, herring, mackerel, lantern fish and neritic squids (Gallo-Reynoso 1991; Niño-Torres et al. 2006; Gallo-Reynoso unpublished data). Concentrations of DDT in these organisms from the Gulf of California are scarcely reported. In 1970, a study reported concentrations of tDDT in lantern fish (*T. mexicanus*) from the central Gulf of California ranging from 13 to 79 ng/g (Cox 1970). These values were considered low at that time, compared with other marine fish of which values were commonly >200 ng/g (Cox 1970). Recent data is only available from the Southern California Bight where northern anchovy had tDDT concentrations of 60 ng/g, followed by Pacific chub mackerel of 41 ng/g and Pacific sardine of 34 ng/g (Jarvis et al. 2007). Although considered different populations with some limited interaction due to partial geographic isolation, these same species of sardine and mackerel occur in the Gulf of California (Lluch-Cota et al. 2007). Due to this geographic separation, it is likely that pesticide concentration differ considerably. Therefore, it is important to investigate the concentrations of persistent organic compounds in lower trophic levels of the Gulf of California food chain. This investigation would provide insight into understanding the biomagnification of these compounds in marine mammals.

It is commonly known that organochlorine concentrations increase with age in cetacean males and sexually immature females due to bioaccumulation in their tissues (Borrell and Aguilar 1987). On the other hand, concentrations of organochlorine compounds in sexually mature females decrease or remain fairly constant due to a net loss through transplacental transfer and lactation of newborn dolphins (Ridgway and Reddy 1995). Although no statistical differences were found due to small sample sizes, we observed one adult male with DDE concentration of 20.8 µg/g lipid wt and another adult male with 10 µg/g lipid wt (Table 1). As age in years (i.e. derived from teeth) was not obtained, we can qualitatively assess gender based on the size of the organism (Vidal and Gallo-Reynoso 2012). The male with the greatest DDE load was also the largest and more likely to have been older than the other adult males. As a result of bioaccumulation, an older dolphin such as this large male, would likely have a higher concentration DDE in its tissue. Juvenile males had the lowest concentrations of all groups (median = 11.5 µg/g lipid wt). These animals are often excluded from the main group (J. P. Gallo pers. observation) and could have less access to food resources compared to adult males, females and juvenile females. Juvenile females presented high median concentrations of DDE (17.3 µg/g lipid wt), as well as the highest individual DDE concentration of all samples (87.3 µg/g lipid wt), indicating high bioaccumulation in these group. The measured DDE concentrations of adult females were only slightly lower than adult males and juvenile females (Table 1). It is possible that not all adult females were active breeders, thus, their DDE burden was not purged through transplacental transfer and lactation.

Endocrine disruption (ED) has recently been recognized as a result of exposure to a variety of chemicals, including DDTs metabolites found in the environment (Colburn et al. 1996). These chemicals are especially harmful during embryonic, foeatal and early postnatal periods as they mimic and/or interfere with hormones, growth factors and neurotransmitters (Colburn et al. 1996). Marine mammals could be at a greater risk to ED due to the bioaccumulation of some of these ED-causing chemicals. The common dolphin population of the Mediterranean has drastically decreased since the 1960s (Bearzi et al. 2003). One possible explanation is the ED caused by DDTs and PCBs found in remnant common dolphin individuals of the Mediterranean (Fossi et al. 2003). We recommend continued monitoring of blubber biopsies from Long-beaked common dolphins to measure concentrations of ED-causing chemicals (including all organochlorine pesticides and PCBs) by applying the methodology proposed by Fossi et al. (1992) to determine the potential health effect of these chemicals in the organisms.

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