Bull Environ Contam Toxicol (2010) 84:731–737
DOI 10.1007/s00128-010-0025-x

Organochlorine Residues in South American Sea Lions, *Otaria flavescens* (Shaw, 1800): Bioaccumulation and Time Trends

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Received: 7 October 2009 / Accepted: 22 April 2010 / Published online: 11 May 2010

Abstract Blubber from stranded South American sea lions (*Otaria flavescens*) was sampled between 1991 and 2005 on the Peninsula Valdés in Argentina and analyzed for organochlorine (OC) pollutants. Mean blubber concentrations, expressed on an extractable basis, were 686 (SD = 1,060) ng g^{-1} for dichlorodiphenyl trichloroethane (tDDT) and 735 (SD = 787) ng g^{-1} for polychlorinated byphenils (PCB). The OC levels were well below those associated with adverse sublethal effects and lethality in mammals. OC concentrations showed statistically significant associations with age that were positive in males and negative in females. These trends are consistent with the majority of marine mammal populations studied. There were no trends in the levels of tDDT or PCB over time. In spite of the low levels detected, OC contamination was present consistently over the 14-year period, suggesting continuous inputs from geographic redistribution.

Keywords PCB · DDT · Pollution · *Otaria flavescens*

In the last several decades, marine pollution has become an increasing problem affecting species and populations of marine mammals. Among many pollutants of diverse origin and nature, organochlorine (OC) compounds have been of great concern for their occurrence in high concentrations even in remote ecosystems despite bans on production and usage. Organochlorine compounds were introduced into the environment after World War II. Because of their extensive use in agriculture and industry, chemical stability and slow biodegradation, these compounds soon became ubiquitous pollutants, particularly in marine environments. Among organochlorines, DDT and PCB are the most widespread and reach the highest concentrations in biota. Their production peaked between 1960 and the late 1970s, and although they are still used in certain areas for limited applications, overall use has been restricted since the late 1970s.

These compounds accumulate in lipid-rich tissues and build up along food webs. Therefore, they preferentially affect fat-rich predators, such as marine mammals in general and pinnipeds in particular. Reported effects of organochlorine compounds on pinnipeds include skeletal deformities and growth alteration, reproductive impairment and immunosuppression. Evidence of a relationship between epizootics in marine mammal populations and organochlorine pollution suggests that OC pollutants are potentially significant contributors to mortality levels (Aguilar and Borrell 1994). Given that organochlorines are bioaccumulative and amplify through food chains, mobile—although resident—top predators such as marine mammals have been proposed as potential bioindicators of the distribution of OCs (Aguilar et al. 2002). However, information on organochlorine concentrations in marine mammals is not homogeneously distributed, either in space or time (Aguilar et al. 2002). The majority of studied populations inhabit the northern hemisphere, principally in European and North American waters, while those from South America have been poorly investigated (Borrell and Aguilar 1999). To the best of our knowledge only one pinniped population (Fillmann et al. 2007) and a few cetacean populations have been analyzed for OCs in South America (Borrell and Aguilar 1999).

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The South American sea lion ranges from northern Peru (in the Pacific Ocean) to southern Brazil (in the Atlantic Ocean). In Argentina, this species spreads along the whole length of the coastline, but concentrates and breeds primarily in continental and island colonies in Patagonia (Dans et al. 2004).

This study has two purposes: The first is to describe the pattern and intensity of DDT and PCB bioaccumulation in Patagonian South American sea lions. The second objective is to determine whether environmental levels of DDT and PCB in the Argentinean waters of Patagonia have changed significantly over the last 15 years via analysis of concentrations in the blubber of the South American sea lion.

Materials and Methods

Between 1991 and 2005, we collected 67 blubber samples from South American sea lions stranded in settlements near the Peninsula Valdés (Northern Patagonia, Fig. 1). From them, 49 were selected because of their good state of freshness and the remaining 18, with a lower condition, were discarded. Once collected, tissue samples were wrapped in aluminium foil and preserved in deep freeze until analysis. For each specimen, body length and sex were recorded and teeth were collected to determine individual ages. Figure 2 details the frequency and ages of males and females sampled each year.

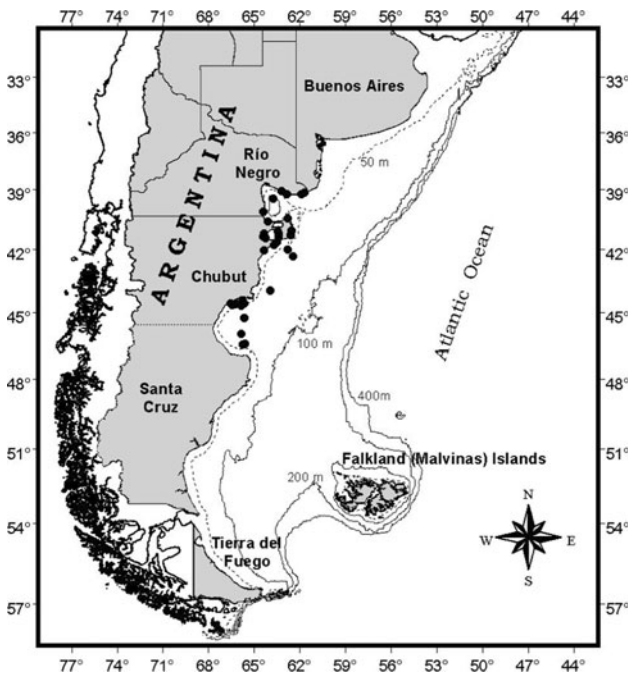

Fig. 1 Sampling location of the South American sea lions

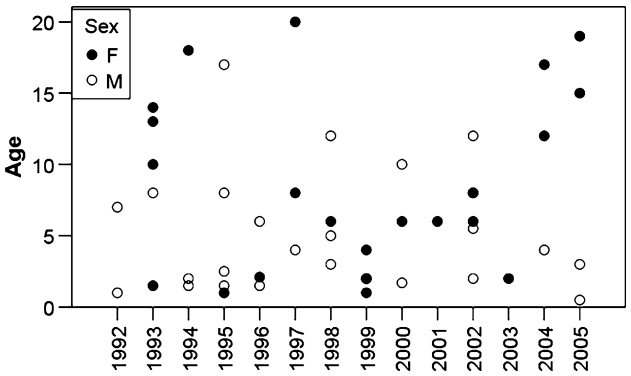

Fig. 2 Frequencies and ages of males and females sampled each year

Blubber samples weighing about 2 g were ground with anhydrous sodium sulphate and extracted with n-hexane (residue-free quality) for 5 h in a Soxhlet apparatus. The solution obtained was concentrated to 40 mL, of which 10 mL were used to determine the amount of extractable fat per gram of blubber. The remainder was mixed with sulphuric acid to clean up the lipids. It was then centrifuged for 5 min to aid separation of the hydrolyzed lipid from the solvent extract.

Instrumental analysis was performed by capillary gas chromatography-high resolution mass spectrometry. A GC8000 Top gas chromatograph (Carlo Erba, Milan, Italy) equipped with a 30 m DB5 capillary column with an ID of 0.25 mm and a film thickness of 0.25 μm was used for the measurements. The temperature program was as follows: 60°C (hold 1 min), an increase to 325°C at 10°C min^{-1}, and a final hold of 20 min. Helium was used as the carrier gas at a constant flow of 1 ml min^{-1}, and 0.5 μL of the sample was injected by an auto-sampler in splitless mode (40 s). Mass spectrometry (Fisons MD800 Thermo Finnigan) was performed in electron impact mode and selected ion monitoring (SIM) was applied for detection. For all compounds, the mass trace of the molecular ion was monitored.

The samples were analyzed for the following compounds: op′DDE (op′, dichloro-diphenyl-dichloro-ethene), pp′DDE (pp′, dichloro-diphenyl-dichloro-ethene), pp′DDD (pp′, dichloro-diphenyl-dichloro-ethane), pp′DDT (pp′, dichloro-diphenyl-trichloro-ethane) and polychlorinated biphenyls (PCB). The tDDT concentration was calculated as the sum of the four DDT compounds. %DDE/tDDT was calculated as the percentage of pp′DDE with respect to tDDT. The total PCB concentration (PCB) was calculated as the sum of seven individual peaks known as ICES7: CB 28, CB 52, CB 101, CB118, CB 138, CB 153 and CB 180. These congeners have been selected by the Community Bureau of Reference as indicators of PCB contamination on the basis of their wide range of chlorination and of their

relatively high concentrations in technical PCB mixtures and in the environment. Aroclor 1254 equivalents were calculated by multiplying the sum of the ICES 7CBs by 2.5 to give an approximate total concentration for a direct comparison with other estimates in Table 1.

Since OCs are highly nonpolar compounds, concentrations in this paper are expressed in parts per billion (ng g^{-1}), calculated on the basis of the weight of the extracted lipids (lipid basis). Blanks of pure n-hexane were run daily during the analysis to ensure the purity of the system. The recoveries of OCs were calculated by adding known amounts of standard to 12 homogenized replicates of the same sample; recovery levels ranged from 82 to 101%. The laboratory participated in interlaboratory calibration exercises for OCs in biota, organised by Quasimeme (1998) and NIST/NOAA (2000 and 2003). Data from our laboratory were in good agreement with those for reference materials.

The detection limit was 1 μg kg^{-1} lipid weight. Values below the detection limits were treated as zero values. All OC concentration data were log10-transformed to achieve normality and increase the homogeneity of variances. Analysis of Student's *t*-tests was used to compare mean concentrations of OCs between males and females. Differences were considered significant at $\alpha = 0.05$. For each sex, the General Linear Univariate Model (GLUM) procedure was used to ascertain the linear relationships between the different contaminants and the ages and sampling years of the specimens, as well as any interaction effects. All statistical calculations were carried out using the SPSS-12 statistical package.

Results and Discussion

OCs pollutant concentrations in the blubber of South American sea lions ranged from 59 to 6,154 ng g^{-1} lipid for tDDT (mean ± std: 686 ± 1,060) and from 27 to 3,770 ng g^{-1} for ICES7-CB (mean ± std: 735 ± 787). CB 153 (IUPAC number) was the most abundant congener, followed by CBs 138, 180, 118, 101, 52 and 28, with the last three being below the detection limit in many cases (30%). This PCB pattern is characteristic of pinnipeds and other marine mammals; congeners 153, 138, 180 are dominant, are normally found in this order, and account for nearly 40% of the tPCB load in top predators.

Table 1 shows tDDT and PCB levels from diverse species of the family Otariidae in different regions around the world. As we can observe, the concentrations of tDDT and PCB detected in the blubber of South American sea lions from Patagonia were relatively low compared with most other otaridae populations. In particular, they were much lower than those found in the Northern hemisphere, such as North Pacific populations in California or Alaska (Table 1). Only the South African fur seal (*Arctocephalus pusillus*, from Namibia) showed lower OC levels than *O. flavescens*.

The South American fur seal from Brazil (*Arctocephalus australis*) is the only pinniped in South America previously analyzed for OCs (Fillmann et al. 2007). In comparison with present data, we can observe that levels from Brazilian samples are between four and seven fold higher than those from Argentina. These dissimilar results could be explained by differences in industrial and agricultural activities between the two zones. Thus, the higher concentrations of OCs in Brazilian individuals would imply the presence of significant sources around the southern Brazilian coastline, possibly arising from significant OC use in the area drained by the Plata River and Los Patos Lagoon (Menone et al. 2001). The low levels observed in South American sea lions could be explained by at least two factors. First, although South American sea lions sporadically relocate to remote waters (e.g., Uruguay) (Szapkievich et al. 1999), their basic area of residence is relatively pristine (Fig. 1). In this region, no significant inputs of these pollutants exist now, nor did they in the past. Second, the marine currents that reach this area, and could thus transport in pollutants from other regions, come from Southern Ocean, where organochlorine compounds have never been released. Presumably, the main source of pollution in this area is atmospheric transport and deposition from more polluted northern areas.

Apart from otariidae, the OC levels found in Patagonian *O. flavescens* were lower than in nearly all other pinniped species, such as the majority of harbour seal (*Phoca vitulina*) populations of the Northern Hemisphere (Aguilar et al. 2002).

Although susceptibility to harmful pollutants varies among species, the levels detected here are considered to be insufficient to produce adverse sub-lethal or lethal effects in the studied population. In fact, they are lower than the thresholds for which effects have been observed in marine mammals (e.g., Kannan et al. 2000), and they are well below the threshold levels at which pollutant-related effects have been observed in other mammals (Forsyth 2001).

Mean pollutant values (log transformed) were significantly higher for males than females ($p < 0.05$) in the whole sample for both PCBs and tDDT. However, they were indistinguishable in individuals younger than 6 years old, and differences were improved in older individuals ($p < 0.005$).

The relationships between age and blubber OC levels in both sexes are shown in Fig. 3. The results from the linear regression model are shown in Table 2. Males and females

Table 1 Concentrations of tDDT and PCB in samples from selected otariidae species from different regions

Species	Locality	Year	Sex	n	% Lipid	tDDT	PCB	Reference
Zalophus californianus californianus	*Southern California*	1994–2006	63f, 29m	92	55 ± 32	594,380 ± 1,670	86,550 ± 263,180	Blasius and Goodmanlow (2008)
	Central California	1993–2002	m	16	45 ± 29	380,000 ± 480,000	77,000 ± 79,000	Ylitalo et al. (2005)
			f	60	34 ± 23	250,000 ± 440,000	83,000 ± 160,000	
	Southern California	2000	8f, 22m, 6u	36	50 ± 24	143,000 ± 257,000	44,000 ± 78,000	Le Boeuf et al. (2002)
	Baja California, México	2000–2001	m	16	59 ± 18	4,170 ± 1,390	3,570 ± 2,100	Del Toro et al. 2006
			f	2	84 ± 4	2,520 ± 900	1,900 ± 990	
Callorhinus ursinus	*Japan*	1972–1998	f	35	64 ± 21	10,330 ± 14,500	5,920 ± 5,610	Kajiwara et al. (2004)
	Alaska St George Is.	1995–1996	m	10	71 ± 10	4,770 ± 1,950	3,030 ± 1,070	Loughlin et al. (2002)
	Alaska St Paul Is.	1995–1996	m	10	60 ± 12	3,390 ± 980	2,400 ± 850	
Eumetopias jubatus	*Alaska US*	1976–1978	m	12	74 ± 12	7,600 ± 5,400	17,000 ± 12,000	Lee et al. (1996)
			f	17	82 ± 5	2,400 ± 2,300	5,300 ± 5,400	
Arctocephalus pusillus pusillus	*Namibia*	1997	m	6	?	366 ± 383	155 ± 267	Vetter et al. (1999)
			f	5	?	168 ± 146	44 ± 37	
Arctocephalus australis	*South Brazil*	1999	3m, 5f	8	32 ± 27	2,100 ± 6,250	7,800 ± 3,750	Fillmann et al. (2007)
Otaria flavescens	*Patagonia Argentina*	1992–2006	m	25	66 ± 21	854 ± 1,192	2,283 ± 2,293	Present study
			f	24	67 ± 19	511 ± 893	1,371 ± 1,465	

All samples are from blubber. Concentrations are expressed in ng g^{-1} on a lipid basis, and were calculated from original data when not directly expressed in the original source. PCB results of *O. flavescens* are expressed as Arochlor 1254. u = unknown

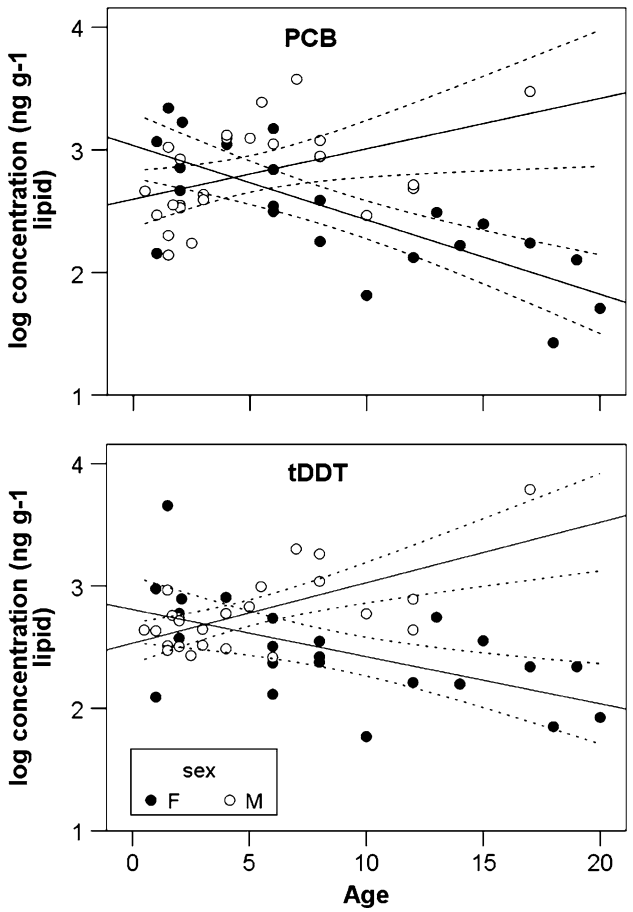

Fig. 3 Regression of PCB (sum of ICES7) and tDDT concentrations with age in blubbers of male (*open circle*) and female (*filled circle*) of South American sea lions

exhibited similar concentrations until they reached sexual maturity at 4–5 years old for females (Crespo 1988). Then, concentration trends diverged for the remainder of their lives. Concentrations in males increased with age, suggesting a pattern in which the rate of absorption exceeded excretion throughout life. In females, the first gestation takes place in this species at 5–6 years of age. Concentration data show an apparent decrease from 5 years of age probably due to the reproductive transfer of pollutants to their offspring via the plancenta and, more importantly, lactation. Many, although not all (Le Boeuf et al. 2002), studies have shown similar general relationships between life history parameters and OC concentrations in pinnipeds (e.g. Hutchinson and Simmonds 1994) and other mammals.

As we saw in the previous section, age and sex affect OC concentrations. Any trend over time in both parameters could have a strong influence over OC trends over time. To remove anomalies in sampling homogeneities that could interfere with OC time trends, age patterns over time for both sexes were analyzed. Figure 2 shows the frequencies

Table 2 Results from the general linear univariate model for each sex and compound type (tDDT and PCB)

	logPCB		logtDDT	
	Males (n = 24)	Females (n = 23)	Males (n = 24)	Females (n = 23)
Model R^2	0.131	0.515	0.385	0.252
Significances				
ANOVA	0.088	0.0001	0.002	0.021
Age	0.029	0.00001	0.001	0.007
Date	0.880	0.476	0.433	0.817
Intercept	0.934	0.527	0.381	0.762

and ages of males and females sampled in different years, demonstrating a randomised sampling. Moreover, regression analyses between age and year for each sex failed to show any relationship between the variables ($p > 0.2$).

tDDT and PCB concentrations in Patagonian South American sea lions examined from 1992 to 2005 displayed no trends over time (Fig. 4). Table 2 presents statistics obtained from the General liner model including age and date of sampling as covariables; the date of collection, contrarily to individual age, did not significantly predict OC concentrations in either males or females.

Most studies focusing on temporal trends in OCs have identified a decreasing trend in recent decades in response to regulations banning or phasing out these chemicals. Since the mid-1970s, levels of tDDT and PCBs have decreased in tissues of marine mammals in many regions around the world. However, Aguilar et al. (2002) indicated that while highly polluted areas show a declining trend in OC concentrations due to proximity to point source discharges, the opposite trend was detected in regions far from these sources, as atmospheric transport and redistribution bring contaminants to less polluted regions (e.g., the systematic long-term transfer of airborne pollutants from warmer to colder regions).

The steady-state concentrations in tissue samples from South American sea lions observed in this study suggest that recent PCB and DDT inputs due to geographic redistribution may be large enough to offset the potential decrease in OC in the tissues due to reduced OC release. Blasius and Goodmanlow (2008) observed the opposite trend in OC concentrations in California sea lions (*Zalophus californianus californianus*), reinforcing Aguilar et al. (2002). In a long term study similar to the present one (from 1994 to 2006), they found declines in both tDDT and PCB. This species, which lives and reproduces in California, is highly polluted with OCs, reflecting the fact that the coasts of California are among the most polluted by OCs in the world (Aguilar et al. 2002).

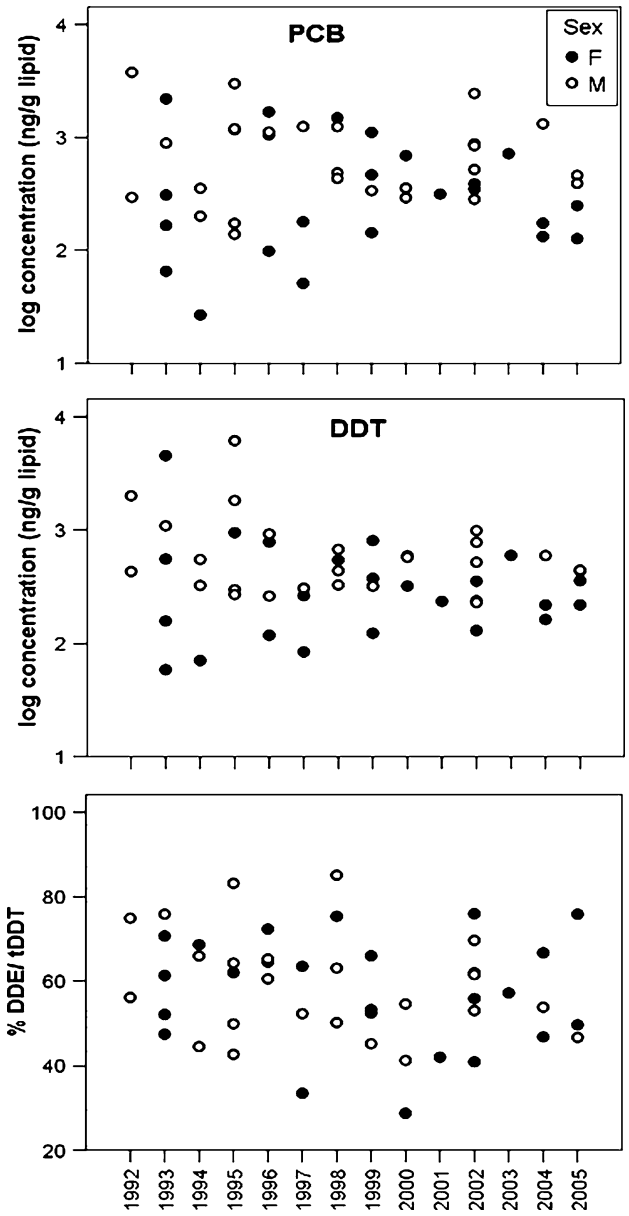

Fig. 4 Trends over time for concentrations of PCB (sum of ICES7) (*upper*), tDDT (*middle*), and the ratio DDE/tDDT (*lower*) in blubber of Southern sea lion from 1992 to 2005 (*filled circle* females, *open circle* males)

Besides raw tDDT concentration, the ratio DDE/tDDT is used to assess the chronology of DDT inputs. DDE is the major breakdown/metabolic product of DDT as it degrades, is more persistent than the parent compound and is not included in commercial, technical DDT (Aguilar 1984). In general, a high DDE/tDDT ratio in an organism means that the organism has not recently been exposed to DDT sources. Aguilar (1984) stated that a ratio >0.6 indicates a stable system with no new DDT inputs. Thus, an increase in the ratio DDE/tDDT over time indicates ageing of the DDT present in the environment and a lack of new inputs. For example, during the 1970s and 1980s this ratio rose progressively in odontocetes and pinnipeds in the North Atlantic concomitantly with a decrease in total DDT loads (Aguilar 1984).

In the current study, the %DDE/tDDT ratios determined in South American sea lion blubber ranged from 29 to 85 (Fig. 4), suggesting that DDT residues are derived from both recent and historic contamination. Moreover, the %DDE/tDDT did not significantly correlate with time (Fig. 4). Although DDT was extensively used in Brazil, Argentina and Uruguay primarily between the 1960s and 1980s (PNUMA, 2002), the lack of an increasing of the %DDE/tDDT over time provides new information, pointing to a constant new release probably due to geographical distribution, as mentioned above.

Miglioranza et al. (2003) support this hypothesis. They analyzed soils from Southeastern Argentina and found high levels of tDDT as well as low %DDE/tDDT ratios (4–17%) in all type of soils. However, natural soils presented the highest concentration of tDDT in spite of never having been used for agriculture and consequently never receiving direct DDT application. The authors justified these high levels as resulting from long-range, transboundary transport, mainly from tropical countries where these pesticides are still in use.

Thus, both the tDDT and DDE/tDDT values found in natural soils and the present study are consistent with the hypothesis that 'new' DDT continues to be atmospherically transported from the surrounding agricultural belt and other remote areas, mostly tropical, where DDT is still in use.

Acknowledgments Thanks are due to many people from the Marine Mammal Laboratory (CENPAT who helped with fieldwork and collected tissue samples for this study). Logistic support for field work was provided by Centro Nacional Patagónico. Lourdes Berdier from Serveis Científico Tècnics of the University of Barcelona is gratefully acknowledged for GC–MS technical support and laboratory assistance. This study was possible through funding granted by Fundación BBVA.

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