The potential use of skin biopsies in the study of 2,3,7,8-substituted PCDDs and PCDFs in sea lion (*Otaria flavescens*) from South-Western Atlantic.

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Introduction

The presence of PCDDs and PCDFs in biota, sediment and air-borne particles near urban, industrialised areas of North America and Europe is well known [1], but there has been relatively little investigation into the levels of these pollutants in remote areas.

As top-level predators in the marine food chain and possessing large lipid reserves, marine mammals are susceptible to significant bioaccumulation of lipophilic pollutants. Marine mammals such as cetaceans and pinnipeds have a limited capacity to decompose hydrophobic, persistent chemicals such as PCBs and DDT as compared with terrestrial mammals, and therefore accumulate high levels of these compounds through the food chain [2].

Conservation management and pollution monitoring programmes require detailed knowledge of xenobiotic metabolism and the effect of these pollutants on marine mammalian physiology in order to evaluate the true extent of the threat to marine mammal populations posed by organochlorines. Little research has been directed at establishing the causal links between contaminants and the health status of marine mammals [3].

In order to understand global pollution by organochlorines, however, some areas still remain to be studied; South-western Atlantic countries belong to one such group [4]. To our knowledge, there is no data of PCDD and PCDF levels in marine mammals living in the coast of Argentina.

In spite of there being much literature concerning organochlorine pollution and its adverse effects on marine mammals, particularly cetaceans, studies dealing with pinnipeds such as sea lions are scarce, there being just one paper on persistent organochlorines in Steller sea lions (*Eumetopias jubatus)* from the Bulk of Alaska and the Bering Sea [5].

Skin biopsy can be used for a wide range of chemical and biomarker analyses. Organochlorines and PAHs can be analysed in subcutaneous fat [6, 7]. Studies along these lines provide valuable tools for non-destructive monitoring of PCDD/Fs in marine mammals. On going research into the improvement of biopsy sampling and analytical techniques has the potential to improve biomarker studies in a non destructive way as it allows the same animals to be sampled regularly [8].

Among the objectives of this paper are to provide for the first time baseline data on PCDD and PCDF levels in marine mammals such as sea lions living in Argentina. This paper presents preliminary data on PCDD and PCDF levels from an endangered population of *Otaria flavescens* living in a heavily polluted harbour (Mar del Plata, Argentina) and a control population (Punta Bermeja, Patagonia) measured in skin biopsies as a non-destructive technique. This is in line with the use of non-destructive techniques for monitoring the health status of endangered wildlife populations [9].

Material and methods

Study species, Study areas and Sampling

The species studied was *Otaria flavescens* (order Pinnipedia), the southern sea lion. Its distribution includes the Atlantic and Pacific coasts of South America.

Samples were taken in two different locations in Argentina, Mar del Plata and Punta Bermeja. The Mar del Plata sea lion colony lives in a fenced area inside the biggest fishing harbour of Argentina, Mar del Plata. The water in the harbour is heavily contaminated by oil, organic and chemical materials and waste from fish processing factories. A large percentage of the old sea lions suffer from diseases of the skin and mucous membranes with fur loss and baldness [10]. The Punta Bermeja area (Patagonia) was used as a "control" environment.

Samples were collected in September 1996 and were taken from males of similar weight and age outside the breeding season. The sea lions were sampled on land with a dart with a punch tip shot from a crossbow. The dart with the biopsy sample was recovered by hand. This sampling procedure is less invasive and safer for the animals [11]. The skin samples (about 0.5 g) were divided into cutaneous tissue which was stored in liquid nitrogen and processed in the laboratory for biomarker analysis, and subcutaneous fat which was stored at -20° C until analysed for PCDDs and PCDFs.

Analytical determination

Extraction and clean up were performed as previously described in detail [12]. Basically this consisted of low pressure chromatography on neutral and base-modified silica gel, activated carbon dispersed on glass fibres, silica gel impregnated with sulphuric acid, and Florisil. Three fractions were eluted from the carbon column for each sample. These contained ortho-substituted PCBs, non-ortho-substituted PCBs and PCDD/Fs respectively. Samples between 0.8 and 2.1 grams of subcutaneous fat were used for analysis. Pooled samples were prepared by combining equal portions (0.1 to 0.3 g) of frozen skin biopsies. Each pool sample contained skin biopsies taken from 9 individuals.

Resolution and quantification of PCDDs, PCDFs and co-planar PCBs were performed by HRGC-HRMS using a VG AutoSpec Ultima (VG Analytical, Manchester, UK) coupled to a Fisons Series 8000 (8060) gas chromatograph. A fused silica capillary DB-5 column (60m, 0.25 mm id., 0.25μm film thickness, J&W Scientific, USA) was used. Helium at a column head pressure of 175 Kpa was the carrier gas. A minimum resolution of 10,000 was used when operating with the HRMS instrument.

Results and Discussion

Table 1 presents levels of 2,3,7,8 PCDD and PCDF congeners together with calculated I-TEQs [13] in sea lion skin biopsies from both colonies studied. Total values were calculated assuming that "not detected" is equal to half the limit of detection. There is a noticeable difference between both colonies; all seventeen toxic congeners were detected in animals from Mar del Plata while only 5 congeners were detected in animals from Punta Bermeja.

In animals from Punta Bermeja, total PCDD/F levels were 37.55 ppt on a wet weight basis. PCDDs contributed 44% to the total values. Among the PCDDs, 1,2,3,7,8,9-HxCDD was the congener exhibiting the highest levels. It is also interesting to note that this congener exhibited levels even higher than OCDD which usually presents the highest levels in biological samples. Total PCDFs contributed 56% to total PCDD/F levels. Among the PCDFs the contribution of two congeners, 1,2,3,4,6,7,8-HpCDF and 1,2,3,7,8,9-HxCDF was noteworth.

Table 1. PCDD and PCDF levels in sea lion skin biopsies from Mar del Plata and Punta Bermeja (pg/g wet weight) and calculated I-TEQs. Total calculated values are given in brackets total values when assuming that all values less than the limit of detection are equal to zero.

Isomer	Punta Bermeja	Mar del Plata
2378-TCDD	*0.42	0.26
12378-PeCDD	*0.42	0.89
123478-HxCDD	*1.82	0.48
123678-HxCDD	*1.72	0.42
123789-HxCDD	7.31	0.68
1234678-HpCDD	*1.39	0.86
OCDD	6.47	2.41
2378-TCDF	*4.20	0.81
12378-PeCDF	*2.79	0.21
23478-PeCDF	*3.53	0.41
123478-HxCDF	*4.34	0.34
123678-HxCDF	*2.39	0.47
234678-HxCDF	*2.37	0.50
123789-HxCDF	2.52	1.31
1234678-HpCDF	5.21	0.58
1234789-HpCDF	*3.61	0.192
OCDF	1.53	0.098
TOTAL PCDDs	16.67(13.78)	6
TOTAL PCDFs	20.88(9)	4.92
TOTAL PCDD/Fs	37.55(22.78)	10.92
I-TEQ PCDDs	1.24(0.74)	0.87
I-TEQ PCDFs	1.94(0)	0.57
TOTAL I-TEQs	3.18(0.74)	1.44

(* Detection Limit)

Total PCDD/F levels in animals from Mar del Plata were 10.92 ppt. In this case PCDDs contributed 55% to the total values and PCDFs contributed 45%, unlike the finding reported for Punta Bermeja colony. However the situation could be similar in animals from Punta Bermeja area when total values are calculated assuming that "not detected" is equal to zero, the contribution of PCDDs being 60% in this case and the contribution of PCDFs 40%. Among PCDDs in animals from Mar del Plata, the contribution of OCDD was high, contributing 22% to the total PCDD levels. This situtation differs sharply from that of animals living in the Punta Bermeja area. It is also noteworthy that samples from Mar del Plata had detectable 2,3,7,8-TCDD concentrations in contrast those from sea lions from Punta Bermeja. From among all PCDFs detected 1,2,3,7,8,9-HxCDF was the congener which contributed the most to total PCDF values.

In animals from Punta Bermeja, PCDDs contribute approximately 40% to the total TEQs, being the contribution of the 1,2,3,7,8,9-HxCDD almost the half of this value. In the case of PCDFs, their contribution to total I-TEQ value is 61% of which the highly toxic 2,3,4,7,8-PnCDF contributes half. When values are calculated (assuming that "not detected" equals to 0), the contribution of 1,2,3,7,8,9-HxCDD increases to 70%, the remaining 30% being contributed by 1,2,3,7,8,9-HxCDF with a 24%, 1,2,3,4,6,7,8-HpCDF with a contribution of 5% and 1,2,3,4,6,7,8-HxCDD which makes a minimum contribution of 0.5 % In animals from Mar del Plata these percentages contributions do not vary with limit of detecction

since all congeners were detected. Here PCDDs contributed 61% to total I-TEQ value, making an important contribution the 1,2,3,7,8-PnCDD with a 31% and the 2,3,7,8-TCDD with an 18%. PCDFs contribute with a 39%, with the toxic 2,3,4,7,8-PnCDF contributing almost the half to this percentage.

Many reports have been published on PCDD and PCDF levels in marine mammals such as seals, beluga and polar bears **[14-15]**, but no data are available on southern sea lions. Data from this study are consistent with other studies on marine mammals which indicated that dioxins occur at relatively low levels in marine mammals **[16]**. Studies on PCCD/F levels on seals suggest that the relatively low concentrations of PCDD/Fs found in seals versus their expected high intake from fish support a presumed rapid catabolism or elimination **[14]**. This could contribute to explain the low levels found in the present study. Pollutant levels in a population can vary considerably within the population's range, owing to both regional dietary differences and environmental and oceanographic factors that act to concentrate or dilute pollution inputs **[17]**.

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