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Chlorinated Hydrocarbons in Skin and Blubber of Two Blue Whales (*Balaenoptera musculus*) Stranded Along the Baja California Coast

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Polychlorinated biphenyls (PCBs) and organochlorine pesticides, generically termed as chlorinated hydrocarbons (CHs), are highly persistent contaminants distributed globally throughout the environment (Voldner and Li 1995). As hydrophobic compounds, CHs exhibit a high affinity for lipids, so they tend to accumulate in fatty tissues of marine mammals. For this reason, most analyses of the CHs body burdens in these organisms have been performed in blubber.

Despite the relatively large amount of data concerning CHs in cetaceans, there is a relative lack of information on contaminants in whales, and in particular in baleen whales (Colborn and Smolen 1996). Information on CHs in blue whales is scant, probably because strandings of this species are very rare, limiting the availability of their tissues for analyses *post-mortem*. Furthermore, reports of CHs in blue whale biopsy samples are also scant (Valdez-Márquez 2001), as this method of sample collection for CH determinations, in this species, has not been validated until recently (Gauthier et al. 1997). The strandings of two blue whales along the Baja California coast in 2000 provided us with the opportunity to analyze the concentrations of persistent organochlorines in skin and blubber samples of these organisms.

To our knowledge, the skin has not been used for the determination of CH levels in whales. Recently, Kunito et al. (2002) used the skin of whales for trace metal determinations, and suggested that this tissue may provide useful information on the status of contamination of these marine mammals. In this study, CH analyses in skin and blubber were performed in an attempt to evaluate the potential use of the skin as a matrix to monitor the contamination of blue whales with organochlorines.

MATERIALS AND METHODS

The two blue whales *Balaenoptera musculus* analyzed in this study were stranded at different locations along the Baja California peninsula in June (specimen Bm1) and October (specimen Bm2), 2000. Bm1 stranded at Bahía de La Paz, Baja California Sur (24°21'5'' N, 110°40'9'' W), and had died just before sampling. Specimen Bm2 stranded at Ensenada, Baja California (31°53'8'' N, 116°42'9''

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W), was dead for a longer period before samples were collected. This specimen was not identified as a blue whale until a DNA analysis was performed (Enriquez et al. 2001). The two blue whales, 18 m long, were juvenile males. Skin and blubber samples were taken close to the dorsal fin and were kept frozen at -10 °C in aluminum foil. The skin was differentiated from the blubber by the thin layer that separates them and that resembles a sheet of wet paper (Gauthier et al. 1997).

CH determinations were made at the Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California. The analyses were made according to Kleivane et al. (2000) and Severinsen et al. (2000) with some modifications. Approximately 500 mg of tissue were spiked with 100 µL of the surrogate dibromo-octa-fluoro-benzene (50 ng mL^{-1}). After maceration, lipids were extracted three times with a mixture of 1:1 acetone-hexane, by ultrasonic disintegration. Extracts were combined and concentrated to 5 mL, a 0.5 mL aliquot was taken for a gravimetric determination of the percentage of lipids, and the remaining 4.5 mL were evaporated to near dryness. After diluting the extract to about 1 mL with hexane, 0.75 mL of concentrated H_2SO_4 (Mallinckrodt A.R.) was added for clean-up. CHs were recovered by three extractions of the mixture with 3 mL of hexane. Finally, extracts were concentrated and transferred to about 1 mL of isooctane, and 10 µL of tetra-chloro-meta-xylene (TCMX, 50 ng mL^{-1}) was added. Quality assurance procedures included the analyses of three replicates of a whale blubber standard reference material (National Institute of Standards & Technology SRM-1945; Table 1), certified calibration standards (ULTRA Scientific), procedural blanks and two replicates of skin and blubber samples. The quantification of CH residues was made with a gas chromatograph (GC) (Hewlett Packard HP-6890) equipped with a ^{63}Ni electron capture detector (ECD), an autosampler, and ChemStation 3392A. A capillary column (J & W DB-XLB) of 60 m length, 0.32 mm internal diameter and a film thickness of 0.25 µm was used. The instrumental conditions were as follows: the split/split-less injector temperature was 275 °C and the detector was at 325 °C; 2 µL of sample were injected, and the total time of the run was 93.67 min. The variation coefficients for the concentrations of all compounds in the calibration standards were lower than 10 % and the detection limit of the instrument was 1 pg μL^{-1}. Since the recovery of the surrogate was about 80 %, concentrations in the samples were corrected.

The CHs analyzed were: *p,p'*-DDT (1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane) and its metabolites *p,p'*-DDD, *p,p'*-DDE; α, β, γ, δ,-HCH (hexachlorocyclohexane) isomers; 13 other pesticides (methoxychlor, heptachlor, heptachlor epoxide, dieldrin, endrin, aldrin, endrin aldehyde, endrin ketone, α-chlordane, γ-chlordane, endosulfan I, endosulfan II and endosulfan sulfate); and 41 polychlorinated biphenyl congeners (PCBs), IUPAC numbers 18, 28, 37, 44, 49, 52, 66, 70, 74, 77, 81, 87, 99, 101, 105, 110, 114, 118, 119, 123, 126, 128, 138, 149, 151, 153, 156, 157, 158, 167, 168, 169, 170, 177, 180, 183, 187, 189, 194, 200, 206 (Ballschmiter and Zell 1980). Results are reported as the sum of analytes within each group (i.e., ΣDDT, ΣHCH, Σpesticides and ΣPCB).

Table 1. Analyses of standard reference material (whale blubber, NIST-1945); n=3.

	Found ($\mu g.kg^{-1}$ wet wt) (mean ± std dev)	RSD* (%)	Certified ($\mu g.kg^{-1}$ wet wt) (mean ± std dev)	RSD* (%)
4,4'-DDD	152 ± 27	17	133 ± 37	28
4,4'-DDE	442 ± 26	6	445 ± 37	8
4,4'-DDT	268 ± 26	10	245 ± 15	6
α-HCH	14.6 ± 2.2	15	16.2 ± 3.4	21
γ-HCH	3.12 ± 0.08	3	3.30 ± 0.81	25
Heptachlor epoxide	10 ± 0.9	9	10.8 ± 1.3	12
Cis-chlordane (α-chlordane)	50 ± 17	34	46.9 ± 2.8	6

*RSD, relative standard deviation

RESULTS AND DISCUSSION

The concentrations obtained for the reference material were within the limits of the certified values (Table 1). Even though the relative standard deviation (RSD) for some compounds was higher than the 10 % obtained for the calibration standards (similar to the RSD of the certified material), this level of precision is expected due to the complexity of the matrix and the methodological errors inherent to the analysis.

The results obtained for CH concentrations in blubber of both whales are, in general, similar to those reported by Valdez-Márquez (2001) for biopsies of 15 living *B. musculus* specimens, including juvenile males, collected in the Gulf of California, between La Paz and Loreto. For example, more than 87 % of ΣDDT was *p,p'*-DDE in both specimens, similar to the average (84 %) obtained by Valdez-Márquez (2001). With respect to ΣPCBs, the relatively low concentrations (< 150 ng g^{-1}) in stranded specimens are within the range reported by Valdez-Márquez, who observed that only 4 of the 15 biopsies were higher than 250 ng g^{-1} lipid wt. Also ΣHCH and Σpesticides concentrations in our blubber samples (Fig. 1) are close to the corresponding ranges (4-184 ng g^{-1} and 275-950 lipid wt, respectively) in living whales (Valdez-Márquez 2001).

Comparing the concentrations in skin and blubber, the four groups of CHs showed a marked concentration gradient with values increasing 4-11 fold from the blubber to the skin of specimen Bm1 (Fig. 1), but such a large gradient between tissues was only clear for ΣDDT in specimen Bm2 (Fig. 1a). The highest CH concentrations were observed for ΣDDT in the skin of both whales, with *p,p'*-DDE being the dominant (70 – 93 %) analyte within this group (Fig. 1a). In contrast with Bm1, which showed a one order of magnitude difference between

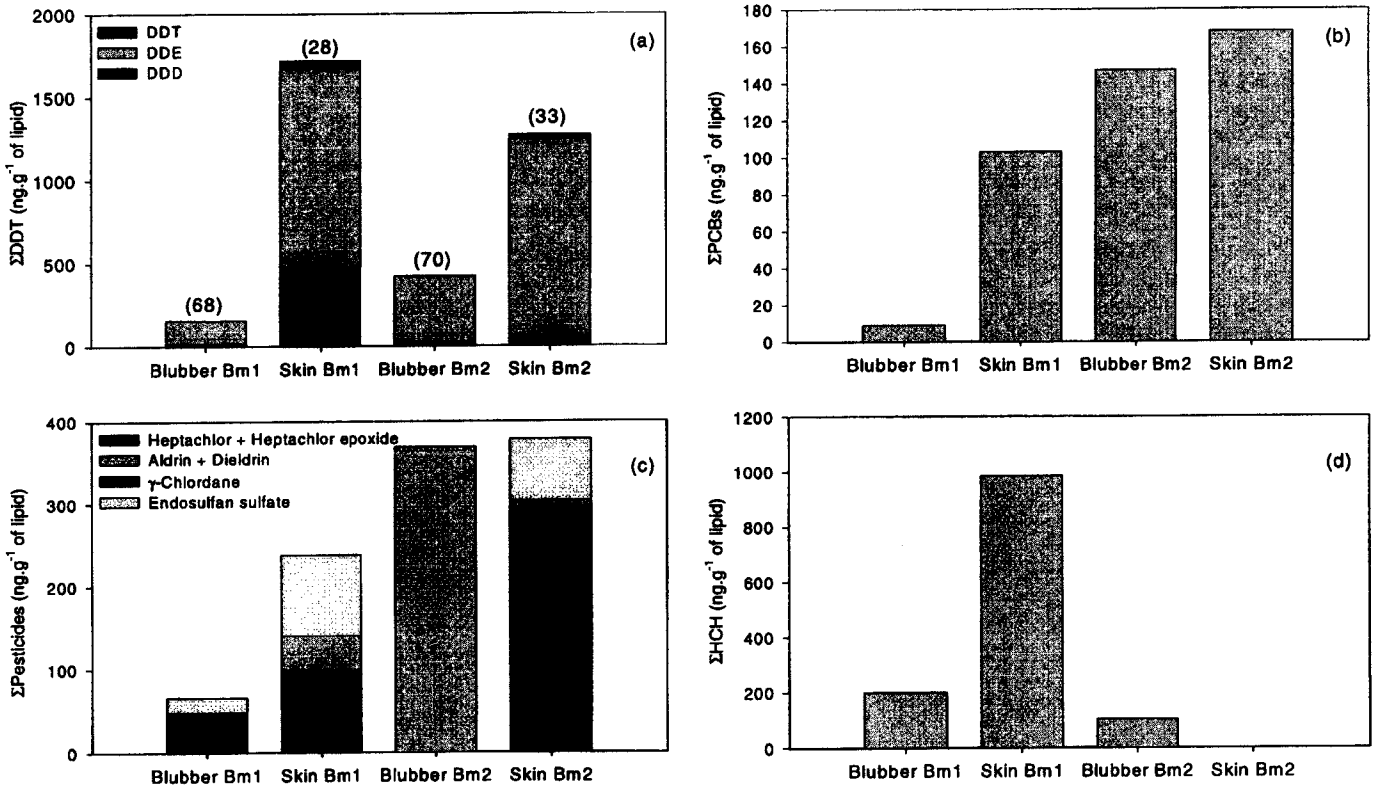

Figure 1. Concentrations of (a) ΣDDT, (b) ΣPCB, (c) Σpesticides and (d) ΣHCH in skin and blubber of the two blue whales stranded along the Baja California coast. Numbers in paranthesis are the percentage of lipids in the samples.

tissues, the ΣPCB concentration was only slightly higher in the skin than in blubber of Bm2 (Fig. 1b). The Σpesticides showed a similar concentration pattern as that shown by ΣPCB (Fig. 1c). By contrast with the other CH groups, although ΣHCH was the second most abundant group in Bm1 tissues, its concentration in the blubber of Bm2 was low and it was undetectable in the skin (Fig. 1d).

The higher CH concentrations found in the skin compared to the blubber of the fresher stranded specimen could be explained by differences in the function and the lipid composition of both tissues, similar to those reported for blubber strata. Studies of CH concentrations in different blubber strata from baleen whales (Aguilar and Borrell 1991) and other marine mammals (Severinsen et al. 2000) commonly show that the outer layer has higher concentrations than the inner layer. These studies support the suggestion by Lockyer et al. (1984; 1985), that the outer blubber zone near the skin is catabolically more stable and represents the main region for long-term energy storage, while the inner blubber is a dynamic region in which fat storage is controlled by nutritional condition and is in a constant state of flux. This difference in metabolic function reflects, in turn, a different composition of fatty acids (Lockyer et al. 1984), with a predominance of short-chained monounsaturated fatty acids in the outermost blubber layer being consistent with its thermoregulatory role (as melting point decreases with decreasing fatty acid chain length; Severinsen et al. 2000). The migration and accumulation of non-polar compounds such as CHs towards the external blubber layer does not, therefore, reflect a simple partitioning of these compounds towards less polar phases, but, as indicated by Aguilar and Borrell (1991), it is linked to the distribution, metabolism and mobilization of blubber lipids.

Our results suggest a preferential accumulation of CHs in the skin, having only 30 % lipid content, when compared with the outer blubber, having around 70 % of lipids (Fig. 1). However, the lack of knowledge on the lipid composition of the skin does not allow us to speculate about the role of partitioning versus metabolic concentration as the mechanism responsible for skin CH enrichment. Our data suggest that the degree of decomposition of the stranded whale had a significant effect on CH composition of the skin, making it necessary to perform more studies to establish comparisons between these tissues. If this preferential accumulation is corroborated, the inclusion of skin tissue in the analysis of CHs in dart biopsy samples of *B. musculus* may cause an overestimation of the CH content in blubber samples.

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