Chlorinated Hydrocarbon Concentrations and Their Use for Describing Population Discreteness in Harbor Porpoises from Washington, Oregon, and California

JOHN CALAMBOKIDIS
Cascadia Research Collective
218½ W. Fourth Avenue
Olympia, WA 98501

JAY BARLOW
Southwest Fisheries Center
P.O. Box 271
La Jolla, CA 92038

ABSTRACT

Concentrations of PCB (polychlorinated biphenyls, a class of industrial chemicals), DDE (the primary breakdown product of the pesticide DDT), and HCB (hexachlorobenzene, a fungicide) were determined in blubber samples from 45 harbor porpoises collected along the coasts of Washington, Oregon, and California. The primary purpose of this study was to test for regional patterns in the concentration of contaminants and their ratios in order to evaluate the feasibility of using contaminants to gain information about the degree of intermixing of harbor porpoises along the west coast of North America. Concentrations varied widely with averages of 14 ppm (mg/kg by wet weight) PCBs, 31 ppm DDE, and 0.51 ppm HCB. Concentrations of contaminants were strongly associated with latitude (location), length of the animal, and sex. Distinct regional patterns were found in both the concentrations of DDE and the ratios of PCB to DDE and HCB to DDE. Contaminant ratios were far less variable than individual contaminant concentrations and were, therefore, more useful for examining regional patterns. Through discriminant analysis using a combination of pollutant ratios, the state (California, Oregon, or Washington) in which harbor porpoises were collected could be correctly predicted for 86% of the samples. Pollutant ratios did not reveal specific boundaries for stocks but indicated that harbor porpoise movements may be restricted in some areas.

Introduction

Chlorinated hydrocarbon contaminants have been recovered from marine mammals from around the world (Gaskin et al. 1971; Taruski et al. 1975; Clausen et al. 1974; Wagemann and Muir 1984). Nearshore marine mammals such as pinnipeds and some cetaceans tend to accumulate high concentrations of stable chlorinated hydrocarbons because they 1) are long lived, 2) feed high on the food chain, and 3) have blubber layers that serve as stable repositories for these lipophilic contaminants.

Harbor porpoise (Phocoena phocoena) occur primarily in nearshore waters off Europe, Asia, and the east and west coasts of North America (Gaskin et al. 1974). Harbor porpoise populations have declined in many parts of their range (Otterlind 1976; Prescott and Fiorelli 1980; Calambokidis et al. 1984), and there is evidence of high rates of mortality in nets along the California coast (Deiter 1991; Diamond and Hanan 1986; Hanan et al. 1986). Estimates of harbor porpoise population size along the west coast of the United States have recently been completed (Barlow 1988) but there is little information on the presence of different population stocks or interchange among areas.

The potential utility of chlorinated hydrocarbon concentrations or ratios for examining movements and intermixing in marine mammals has been reported previously (see review in Aguilar 1987). Winn and Scott (1978) included differences in PCB and DDT concentrations as part of the
Table 1

Sources of harbor porpoise samples analyzed in this study.

<table>
<thead>
<tr>
<th>Sources</th>
<th>Region</th>
<th>Years collected</th>
<th>No. of samples</th>
<th>Sample prefix</th>
</tr>
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<td></td>
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<td>1984-86</td>
<td>8</td>
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<td>18</td>
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The purposes of this study were to 1) determine the concentrations of PCBs (polychlorinated biphenyls, a class of industrial chemicals), DDE (the primary breakdown product of the pesticide DDT), and HCB (hexachlorobenzene, a fungicide) in harbor porpoises from Washington, Oregon, and California; 2) test for regional differences in these contaminants and their ratios; and 3) evaluate the utility of this information in discerning harbor porpoise populations.

**Methods**

Blubber samples from 45 harbor porpoises were tested for concentrations of PCBs, DDE, and HCB. Also, seven blubber samples were taken from different locations (anterior-dorsal, mid-dorsal, posterior-dorsal, anterior-ventral, mid-ventral, posterior-ventral, and mid-lateral) from each of two harbor porpoises for testing to evaluate toxicant differences based on body location.

**Sample Collection**

Samples for analysis were received from a wide variety of sources in addition to those collected by the authors (Table 1). All were collected from animals found dead on the shores of Washington, Oregon, and California (Fig. 1). Samples were stored either in glass, aluminum foil, or plastic bags. Samples were stored frozen after collection, except those provided by the California Academy of Science, which had been preserved in formalin. Coordinating organizations also provided information that was tested for association with contaminant concentrations, including collection location, date, sex, length, and blubber thickness.

**Sample Analysis**

Analyses for concentrations of PCBs, DDE, and HCB were conducted as described in previous reports (Calambokidis et al. 1979b, 1984; Mowrer et al. 1977). The analyses were conducted at the Environmental Analysis Laboratory of The Evergreen State College.

Approximately 5 g of blubber, subsampled from the unexposed interior of samples received, was digested in 50 mL BFM solution (glacial acetic and perchloric acid) over a steam bath for several hours (Stanley and LeFavoure 1965). Samples were extracted four times with 20 mL aliquots of "pesticide quality" hexane. Lipid weights were determined by evaporating a portion of the hexane-lipid extract to dryness. A 10 mL portion of the hexane-lipid extract was cleaned with 1-2 mL concentrated sulfuric acid (Murphy 1972). After centrifuging, 1-9 µL was injected into a Hewlett-Packard electron capture (63Ni) gas chromatograph equipped with a 1/4" x 6" glass column packed with 10% DC-200 on Gas Chrom Q, 80/100 mesh. The column also had a 1" alkaline (KOH and
NaOH) precolumn to reduce interference from other compounds and to convert any small amounts of p,p'DDT to p,p'DDE (Miller and Wells 1969). The concentration of p,p'DDE plus any p,p'DDT will be referred to as DDE throughout this paper.

Contaminants were identified and quantified based on comparison of elution times and peak areas to PCB, DDE, and HCB standards injected daily. PCBs (a mixture of compounds) were quantified by individual homolog analysis using mean weight percent figures reported by Webb and McCall (1973). Minimum PCB values were calculated using only the more chlorinated PCB homologs corresponding to the PCB components present in the commercial PCB mixture Aroclor 1260. Though additional less chlorinated PCB homologs were present, they were not included in the total because some samples contained interfering compounds and a reproducible minimal value was considered more important than a more variable estimate of total PCBs. The magnitude of this downward bias is approximately 25–40%.

Multiple linear regression and ANOVA were used to evaluate the association between contaminant concentrations and other variables. Concentrations based on lipid weight were used for the linear regressions because lipid weight was found to be significantly correlated to concentrations in models using wet weight. Concentrations were log transformed for these calculations to meet the assumptions of normal distribution of data. In addition to latitude, collection location was categorized by state and five collection locations: the Morro Bay, CA area \((n = 2)\); the Monterey Bay, CA area \((n = 13)\); the San Francisco/Bodega Bay, CA \((n = 9)\); Oregon \((n = 13)\); and Washington \((n = 8)\). (More detail was included in California owing to high fishery mortality there.) Two samples were excluded from multivariate tests: one collected 10 years prior to the other samples and the other collected within the inland waters of Puget Sound, Washington.

Stepwise discriminant analysis and stepwise multiple regression were used to determine whether collection locations could be predicted from linear combinations of pollutant values. Discriminate analysis was formed using collection location as the categorical variable. A jackknifed calculation system was used to determine the predictive power of discriminant functions (Lachenbruch and Mickey 1968). Each sample was classified based on discriminant functions calculated from all data excluding the sample being classified. Multiple regression was performed using latitude of the recovered sample as the dependent variable. Five predictive variables were considered (all expressed as ratios): PCB/DDE, HCB/DDE, PCB-14/PCB, PCB-16/PCB, and PCB-17/PCB. The PCB-14, PCB-16, and PCB-17 components represent homologs of PCB that comprise a portion of the total PCBs quantified. The DDE was chosen as the denominator for the first two variables because it showed lower coefficient of variation than did PCBs or HCB. Because the variables were expressed as ratios, the assumption of homogeneity of variance is violated. For this reason, more emphasis will be placed on the descriptive aspects of multivariate analyses, and little emphasis will be given to significance tests. Multivariate and discriminant statistical tests were performed using SYSTAT (Wilkinson 1986) and BMDP (Dixon 1985) computer programs.

**Results**

Concentrations of PCBs, DDE, and HCB in the blubber of the 45 harbor porpoises examined varied widely (Tables
The DDE concentrations tended to be higher, averaging wet weight, SD and length was not consistent for males and females. For effects were generally weak or not significant in all models. Models, with higher concentrations associated with thinning between PCBs and animal length.

31 ppm (wet weight, SD = 2.0) were found. A significant regression was found between PCB concentrations and animal length (by lipid weight, SD = 2.078, P < 0.05). Blubber thickness and year of collection significantly influenced contaminant concentrations in some models, with higher concentrations associated with thinner blubber layers and earlier collection years. These effects were generally weak or not significant in all models.

The association between PCB and DDE concentrations and length was not consistent for males and females. For both PCBs and DDE the association between concentrations and animal length was significant in males (n = 17, r = 0.70, P < 0.01 and r = 0.76, P < 0.01, respectively) but not in females (n = 26, P > 0.05 for both PCB and DDE). The significant associations with length in the entire sample, therefore, primarily reflect this association in males only.

Ratios of contaminants were less varied than the concentrations. Both the ratios of PCB to DDE and HCB to DDE varied significantly by latitude (r = 0.70, P < 0.001 and r = 0.83, P < 0.001, respectively). Similarly both these ratios varied significantly among regions (ANOVA, P < 0.001 in both cases). No significant associations were found between ratios and other factors. Figure 2 illustrates differences in the PCB/DDE ratio among regions.

Analyses of blubber samples from seven different locations on two harbor porpoises (14 samples) yielded similar results. In both animals, samples from the dorsal peduncle area had about 20% lower concentrations than other samples. Concentrations from other parts of the body were fairly uniform deviating less than 10% in most cases (never more than 20%) from average values for all samples (excluding the dorsal peduncle). Further details of this comparison are reported in Calambokidis (1986). Concentrations (by lipid weight) and ratios for four samples pre-

### Table 2

<table>
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<th>Latitude (degrees)</th>
<th>Collection date (d)</th>
<th>Sex</th>
<th>Standard length (cm)</th>
<th>Blubber thickness (cm)</th>
<th>% lipid</th>
<th>Concentration (mg/kg, wet wt.)</th>
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<th>DDE</th>
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<td>15 07 1986</td>
<td>F</td>
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<td>92</td>
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Concentrations of PCBs averaged 14 ppm (mg/kg wet weight, SD = 13) or 21 ppm (lipid weight, SD = 23). The DDE concentrations tended to be higher, averaging 31 ppm (wet weight, SD = 30) or 45 ppm (lipid weight, SD = 46). The HCB concentrations were much lower than PCB or DDE averaging 0.51 ppm (wet weight, SD = 0.42) or 0.77 ppm (lipid weight, SD = 0.80). Concentrations of DDE (lipid weight, log transformed) varied significantly among the five regions compared (ANOVA, P < 0.001). No significant differences were found among regions for PCB or HCB (ANOVA, P > 0.05).
by plotting the first and second factor scores for each discriminant analysis, the separation of groups is illustrated (Fig. 3; note that a plot of factor scores would be a simple rotation of this figure). As seen in Figure 3, there are three samples taken in Oregon which appear to be more similar to California samples, and there is one sample from Washington which appears similar to Oregon samples. Within California, the southern samples appear relatively distinct from other areas, but there is considerable overlap between Monterey Bay and areas north of there. Samples from Monterey Bay are characterized by very low variance in both PCB/DDE and HCB/DDE ratios (Fig. 3).

Multiple regression was able to predict accurately the latitude at which samples were collected using 5 variables (multiple $R = 0.89$). Again the fractional components of PCBs did not add appreciably to the regression and were excluded by the stepwise procedure. The regression coefficient (multiple $R$) was 0.87 using only PCB/DDE and HCB/DDE. The predicted and estimated latitudes for each sample are shown in Figure 4. There appear to be four outliers in Oregon which appear more like those from northern California and one sample from California that appears more like those from southern Oregon.

**Discussion**

Concentrations of chlorinated hydrocarbons found in this study were generally in the middle of the range reported from harbor porpoises from other areas (Gaskin et al. 1971; Koeman et al. 1972; Clausen et al. 1974; Otterlind 1976; Calambokidis et al. 1984). Correlations between length and contaminant concentrations found in this study...
Figure 2

Number of samples, mean, and range of PCB/DDE ratios for harbor porpoises from different areas. Excluded are one sample collected in 1971 and one sample from Puget Sound (inland waters of Washington State).

Table 4

Predicted sample locations from discriminant analysis using PCB/DDE and HCB/DDE as predictive variables. Samples were categorized as being from Morro Bay Area (CA-1), Monterey Bay (CA-2), San Francisco/Bodega Bay area (CA-3), Oregon (OR), and Washington (WA). Excluded are one sample collected in 1971 and one from Puget Sound (inland waters of Washington State). A total of 27 lose samples were predicted correctly to region and 37 were predicted correctly to state (out of 43 samples).

<table>
<thead>
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<th>Actual collection location</th>
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<th>CA-2</th>
<th>CA-3</th>
<th>OR</th>
<th>WA</th>
<th>Total</th>
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<td>Total</td>
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<td>11</td>
<td>10</td>
<td>11</td>
<td>7</td>
<td>43</td>
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</table>
are consistent with other reports of accumulation with age primarily in male harbor porpoises (Gaskin et al. 1982; 1983) and other marine mammals (Addison et al. 1973; Addison and Smith 1974; Donkin et al. 1981; Calambokidis et al. 1984).

Organic pollutant residues give valuable clues regarding the population structure and feeding ecology of west-coast harbor porpoises. If the population were panmictic (randomly mixing), homogeneous pollutant ratios for all samples should exist. Instead, very strong gradients occur with latitude. This is likely to occur only if individual porpoises remain in one area for long periods of time. Similarly, pollutant ratios imply something about the feeding ecology of west-coast harbor porpoises. The observed patterns would not be expected if harbor porpoises were feeding on fish populations which had homogeneous pollutant ratios. It is likely that harbor porpoises feed largely on local fish stocks rather than on highly migratory fish. These patterns appear to differ from those of harbor porpoises along the east coast of the United States which may migrate a considerable distance to feed on a migratory fish, the herring, in the Bay of Fundy (Gaskin et al. 1985).

If an individual changes location, it is not known how much time is required to attain pollutant ratios which are characteristic of the new location. The required time period is related to the residence times of the pollutant and the difference between current pollutant load and that characteristic of the new location. Because chlorinated hydrocarbons accumulate over prolonged periods of time (the entire lifespan in males), we infer that most harbor porpoises remain in a region for extended periods if not most of their lives. Some exceptions may be evident in the data. Both discriminant and multivariate regression analyses identified several individuals from Oregon whose pollutant ratios more closely resembled samples from northern California. Although this could be natural variation about a mean value for Oregon, it could also be due to animals that moved at one point during their lives from California to Oregon or to animals that regularly move between those regions.

Pollutant ratios in Monterey Bay samples are particularly interesting because of their low variance. Monterey Bay samples show little variability in both PCB/DDE and HCB/DDE ratios (Fig. 3). Samples collected north of Monterey Bay (primarily near San Francisco and Bodega Bay) overlap with those values seen in Monterey Bay, but have much higher variance. In the discriminant analysis, 62% of the misclassifications were associated with this San Francisco/Bodega Bay area (Table 4). The low variance may be indicative of a resident population in Monterey Bay.

Figure 3
Pollutant residues expressed as the ratios HCB/DDE and PCB/DDE for 43 samples collected in 5 regions. Regions are defined in the text. Polygons enclose all samples collected in each of the 5 regions.
Bay. The higher variance of the more northerly samples may indicate an area of mixing. Although speculative, these interpretations could be tested as additional information becomes available.

Unfortunately, pollutants ratios do not indicate any logical subdivision of the west-coast porpoise population into stock units. A stock is a management term and does not have a widely accepted definition. The Marine Mammal Protection Act of 1972 defines a stock as a group of animals of the same species which inhabit a common spatial arrangement and which interbreed when mature. Perhaps the best definition of a stock is a collection of animals that can be sensibly managed as a single unit (Larkin 1972; MacCall 1984). The problem with harbor porpoise management is that the animals do not appear to fit this concept of a stock. Based on pollutant ratios, harbor porpoises do not move great distances; thus animals from California and Washington are not likely to interbreed and should thus be assigned to different stocks. However, there may be movement from Washington to Oregon and from northern California to Oregon. Harbor porpoise distributions are continuous between California and Washington (Barlow 1988) and there are no apparent barriers to movement or gene exchange. Thus assigning clear boundaries of potentially discrete stocks may not be possible.

It is unrealistic to expect that one technique, analysis of pollutant ratios, will answer all questions about stock structure. We have suggested, however, that harbor porpoise interchange between some areas is relatively restricted. Other techniques, such as conventional tagging or radio tracking may help refine knowledge of their movements and use of cytogenetic and biochemical methods (see Duffield et al. 1991) may determine degree of interbreeding. Until such additional information becomes available, we urge a conservative approach to harbor porpoise management, avoiding depletion of populations in local areas.

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Citations


