



**CHLORINATED HYDROCARBONS IN HARBOR PORPOISE FROM WASHINGTON, OREGON, AND CALIFORNIA: REGIONAL DIFFERENCES IN POLLUTANT RATIOS**

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**John Calambokidis Cascadia Research Collective Waterstreet Bldg., Suite 201 218 1/2 W. Fourth Ave. Olympia, Wa. 98501**

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# <span id="page-6-0"></span>CHLORINATED HYDROCARBONS IN HARBOR PORPOISE FROM WASHINGTON, OREGON, AND CALIFORNIA: REGIONAL DIFFERENCES **IN POLLUTANT RATIOS**

### **John Calambokidis**

#### **ABSTRACT**

**Concentrations of pcb DDF hpr samples collected from <sup>36</sup> harbo? norlo? exara^ned ln <sup>51</sup> blubber**  $%$  **coalfered containable 2011 compose collected** along the **purpose of the study was to test l ™!3, The Primary** purpose of the study was to test for regional patterns in the *concentration of contaminants and their ratios and to evaluate* **the degree of intermixing of** harbor **™ t0- g31n lnformation about** the degree of intermixing of harbor porpoise along the west coast of North America. Strong regional patterns were found in both the concentrations of DDE and the ratios of various contaminants. **contaminant** ratios were far less variable then individual **examining regional patterns ThJ ere/ore more useful for** examining regional patterns. The ratios of DDE/PCB and HCB/DDE showed the most dramatic differences by location with highly  $c$  **correlations correlation correlation containst in the** *terpies* **correlations correlations correlations correlations correlations correlations correlations correlations correlations correlatio** correlation (p<0.001) between the ratio and latitude that the  $s$  amples were collected. Within California samples from 3  $t$  subregions showed significant differences in contaminant ratios, gain information on geographic interchange of harbor porpoise *^ t J t l*  $\frac{1}{2}$  **promising, especially in areas like California** where the presence of pollutants in the marine environment varies widely by

Significant patterns were also found in contaminant concentrations by year collected, animal's length, and blubber thickness. Replicate samples of the blubber taken from different locations on the bodies of two harbor porpoise showed minimal **variation.** Only samples from the dorsal peduncle area of the porpoise deviated from values obtained from other parts of the

### **INTRODUCTION**

bob (the principal environmental motobolity including PCB, **DDT), and HCB have been' dccunantad °f the Pesticide**  $DDT$ ), and HCB have been documented throughout the global ecosystem in the last 20 years. Although use of many of these contaminants has been restricted in some parts of the world including the U.S., their stability and assimilation into marine food chains make them some of the most prevalent contaminants found in marine organisms. Risebrough (1978) reviewed the occurrence and impacts of pollutants in marine mammals. Near-<br>shore marine mammals such as pinnipeds and some cetaceans are the<br>most susceptible to accumulating high concentrations of some of shore marine mammals such as pinnipeds and some cetaceans are the

<span id="page-7-0"></span>these 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 lipophillic contaminants.

Harbor porpoise (Phocoena phocoena) are a primarily nearshore species. Its small size, wary nature, and undramatic behavior has made it a difficult animal to study and consequently there are many aspects of harbor porpoise biology that are not<br>known. In particular there is little informational In particular there is little information on longdistance movements or presence of different population stocks. Concerns over human-induced mortality in certain portions of the harbor porpoise's range has made these questions important for management of harbor porpoise.

Harbor seals in Washington State were found to have distinct ratios of PCB and DDE that were unique to different regions (Calambokidis et al. 1984). Calculations of the total body burdens of PCB and DDE in harbor seals and their prey indicated that these ratios reflected accumulations from years of intake and therefore would remain reasonably constant in an individual for some time. Contaminant ratios may therefore be useful in evaluating the degree of geographic intermixing that occurs in other marine mammals.

The purpose of this study was to determine the concentrations of PCB, DDE, and HCB in harbor porpoise from Washington, Oregon, and California and to test for regional differences in these contaminants and their ratios.

#### METHODS

Fifty two harbor porpoise samples were tested for concentrations of PCB, DDE, and HCB in this study. Of these, 14 were duplicate samples taken from different parts of the body of two harbor porpoises, and one was a blind duplicate provided by the Southwest Fisheries Center. Without duplicate samples, blubber samples from 36 different harbor porpoise from hington, Oregon, and California were examined in this study.

#### Sample collection

Samples for analysis were received from a wide variety of sources. The study would not have been possible had it not been for the uncompensated cooperation and help of these other researchers. Table 1 summarizes the organizations that provided samples for analysis and the time period and regions in which the samples were collected. Samples provided 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.

Cooperating organizations also provided information that was tested for association with contaminant concentrations including: collection location, date, sex, length, and blubber thickness.

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<span id="page-8-0"></span>Locations were used to determine latitude where all samples were<br>collected. Specific locality information was not available and Specific locality information was not available for the two southernmost California samples analyzed. Latitude of these samples was estimated from general information on the locations they were from.

Because of variations in sample collection and storage subsamples for analysis were generally taken from the unexposed interior of the samples, except for a few cases when samples were too small to allow this.

#### Sample analysis

Analyses for concentrations of PCB, DDE, and HCB were conducted as described in previous reports (Calambokidis et al 1979, 1984, Mowrer et al. 1977). The analysis was conducted by Cascadia personnel using the Environmental Analysis Laboratorv at The Evergreen State College.

Approximately <sup>5</sup> g of sample 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'<br>hexane. Lipid weights were determined by expressions 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 up with 1-2 ml concentrated sulfuric acid (Murphy 1972). After centrifuging 1-9 ul was injected on a Hewlett-Packard electron capture (63Ni) gas injected on a Hewlett-Packard electron capture (63Ni) gas<br>chromatograph equipped with a 1/4" x 6' glass column packed with<br>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).

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 later eluting more chlorinated PCB homologs for quantification. This peaks correspond 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 they may have included some additional interfering compounds and results based on these peaks did not appear as consistent. The minimum PCB values were used because for the purposes of this study 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%.

Typical chromatograms from two samples and a PCB standard are shown in Figures 1-3.

#### **RESULTS**

<span id="page-9-0"></span>Results of sample analyses are presented by state in Tables 2-4 and locations where samples are from shown in Figure 4. PCB values ranged from 0.2 to 49.6 ppm (wet wt.). Since these PCB values are minimums as discussed above, the estimated true maximal values were just under 100 ppm (wet wt.). DDE values also ranged widely from .2 to 132 ppm (wet wt.). HCB values were much lower and ranged from .0099 to 1.78 ppm. Concentrations are reported by wet weight instead of a lipid weight basis because no correlations were found between sample concentrations and percent lipids. Ratios of contaminants are identical whether calculated by lipid or wet weight.

Ratios of contaminant concentrations were found to be far less variable that the individual sample concentrations. This reflected the strong correlation between the concentrations of the three contaminants (p<0.05 for all combinations).

## Differences by location

Contaminant concentrations and ratios were tested for differences by location in three ways: 1) testing for differences between states, 2) testing for correlations with latitude, and 3) testing for differences among eight general regions (2-3 regions within each state, see Fig. 4).

Regional and geographic patterns were found in the ratios of contaminants and to a lesser degree concentration of some contaminants. DDE was the only contaminant that varied<br>significantly among states (ANOVA, p<0.025). The difference<br>between ratios of both DDE/PCB and HCB/DDE were highly significant among states (ANOVA, p<0.001). Regional differences were also reflected by the highly significant correlations that were found between latitude and DDE concentration (r=-0.52, p<0.002), latitude and DDE/PCB (r=-0.70, p<0.001), and latitude and HCB/DDE (r=0.81, p<0.001). The relationship between latitude and these three variable is also shown in Figures 5-7. Overall DDE concentrations, and the DDE/PCB ratio was highest in California and lowest in Washington, with intermediate but HCB/DDE ratios were lowest in variable values in Oregon. California and highest in Washington.

Regional differences were also apparent within states (Table 5) especially in the DDE/PCB ratio (Figure 8). The California samples were from three regions: two samples from the vicinity of Morro Bay, nine samples from the vicinity of Monterey Bay, and four samples from just outside San Francisco Bay. The DDE/PCB ratios did not overlap between these three data sets with a single exception. One sample from north of San Francisco Bay had a very different DDE/PCB ratio from the other three samples from this area. This was also the only sample examined that was collected prior to 1980. Since this sample was from 1971, the only sample collected prior to the banning of DDE use in the United States, it is not unexpected that it deviates from the

#### <span id="page-10-0"></span>**regional pattern.**

**Unlike California samples, no patterns within state were apparent in the samples from Oregon. Samples from Oregon showed the greatest variations in contaminant ratios. DDE/PCB ratios of Oregon samples overlapped those from California but were generally higher than samples from Washington. HCB/DDE ratios of Oregon samples, however, were similar to those from Washington but generally higher than those from California.**

**Washington samples were fairly consistent in the ratio of DDE/PCB. With one exception all of the Washington samples had the lowest DDE/PCB ratios of all the samples tested. Within the state there was also an increasing ratio of DDE/PCB with decreasing latitude.**

#### **ionship with .lengthy sex± collection date, blubber thickness, and lipid content**

**Contaminant concentrations and ratios were tested against a variety of other variables to identify other factors influencing the data. These tests were complicated by the broad geographic regions sampled and the lack of a large sample size of consistently collected animals from the same area. Significant correlations between contaminants (and contaminants ratios) were not found with month of collection, sex, or percent lipids of the sample (p>0.05 in all cases, by ANOVA or correlation analysis, as appropriate). Some significant patterns were found with year, length, and blubber thickness. The significance of these associations (or lack of association) should be viewed cautiously because of the limited sample size.**

**Several significant relationships were found with year collected. There was a weak but significant positive correlation between year collected and the HCB/DDE and the HCB/PCB ratios (p<0.05,. both cases). When Oregon and Washington samples were tested independently from the California samples there was a strong negative correlation between year and PCB, DDE, and HCB concentrations (p<0.002 in all cases).**

**Several weak correlations were found with length of animals. There was a positive correlation between PCB concentrations and animal length (p<0.05). Similar positive correlations between length and both PCB and DDE were found when limiting the data to the California samples only (p<0.05).**

**Blubber thickness was significantly positively correlated to PCB concentrations (p<0.05) in the entire data set. Significant positive correlations were also found when the analysis was restricted to Washington-Oregon between blubber thickness and PCBs (p<0.005), DDE (p<0.05), and HCB (p<0.02). A negative correlation was found between blubber thickness and DDE/PCB ratio (p<0.05) again in the Washington-Oregon sample.**

### <span id="page-11-0"></span>Differences by blubber location

Samples used in this study were collected from different locations on the body of the animals. To test for possible biases introduced by sample location, we tested blubber sample ollected from seven locations on the body of two different harbor porpoise. Each group of samples were run together, separately from the analysis of other animals, to try to limit he variations caused by the analysis procedure.

Results of these multiple samples are shown in Tables 6 and<br>7. Overall there was good agreement in both . Overall there was good agreement in both contaminant oncentrations and ratios between the different samples. In both<br>concentrations and ratios between the different samples. In both<br>sets of samples, however, the sample from the dorsal peduncle had sets of samples, however, the sample from the dorsal peduncle had<br>the lowest concentrations. In only one of these two cases were contaminant ratios for the dorsal peduncle also outside the range of the other samples. Body location does not appear to be a major concern in sample collection though the dorsal peduncle<br>should be avoided if possible.

#### DISCUSSION

# Other reports of contaminants in harbor porpoise

Concentrations of chlorinated hydrocarbons in harbor<br>porpoise have been reported by a variety of authors including:

Author Mo. Samples Location



In general contaminant levels found in this study fall in the middle of the wide range of values reported in harbor porpoise from other areas. Unfortunately there are relatively few data available on previous analyses of harbor porpoise along the west coast of North America. O'Shea et al. (1980) reported

<span id="page-12-0"></span>**on PCB, DDT, and HCB concentrations in one harbor porpoise from California though the date or location of collection was not specified. Schafer et al. (1985) reported concentrations of PCB and DDT in coastal bottlenose dolphins from Southern California. Concentrations were much higher than those found in harbor porpoise. The DDE/PCB ratio found in the bottlenose dolphins was also higher than found in this study, though this is consistent with our finding of increasing DDE/PCB ratio at decreasing latitudes approaching Southern California.**

# **Relationships with sex, length, date, blubber thickness, and percent lipids ~**

**The negative correlations between contaminants and year collected would be expected since PCB and DDT have been banned from commercial use and recent reports have indicated a general pattern of decrease in PCBs and DDE in fish and invertebrates along the U.S. west coast (Matta et al. 1986). Positive correlations between length and contaminant concentrations are consistent with observations of higher contaminant concentrations with age in other marine mammals (Calambokidis et al. 1984, Addison and Smith 1974, Addison et al. 1973, Donkin et al. 1981). Gaskin et al. (1982, 1983) found concentrations of DDT and PCBs increased with age in male harbor porpoise but decreased with age in females, probably as a result of loss through reproduction. Duinker and Hillebrand (1979) reported on the transplacental transfer of chlorinated hydrocarbons including PCB, DDE, and HCB in harbor porpoise.**

**It was surprising that a positive correlation was not found between concentrations of contaminants and the percent lipids. This association would be expected to be present since chlorinated hydrocarbon contaminants are stored in the lipids of the blubber layer. Any significant leaching out of oil would be expected to decrease the contaminant concentrations by wet weight. Significance may not have been found in this case because most samples were in good condition with greater than 50% lipids and other factors contributed far more to the variation between sample concentrations.**

**The positive correlations between blubber thickness and contaminants found in this study is somewhat surprising and probably spurious because a negative correlation has been reported in other marine mammals (Calambokidis et al. 1984, Donkin et al. 1981, Addison and Smith 1974). Aguilar (1985) postulated that when blubber is mobilized in cetaceans a portion of the contaminants present is also mobilized but another portion is not and would therefore result in a higher concentration of contaminants in the remaining blubber. This alteration in contaminant concentrations led Aguilar (1985) to conclude that testing of blubber tissues from stranded cetaceans that may have metabolized some or all of their blubber layer during the period prior to death might give biased results of the true contaminants present in the healthy population. This problem is important when comparing contaminant concentrations between stranded and**

<span id="page-13-0"></span>incidentally caught animals but should not be of major consequence in comparing contaminant ratios.

Problems with sampling blubber on different parts of the body of cetaceans have been summarized by Aguilar (1985). concludes that there are likely significant differences in contaminant concentrations in different parts of the blubber of This included both parts of the body and blubber strata at a particular location. These differences were primarily the result of differences in the lipid composition in different parts of the blubber. This sampling problem does not seem to be a problem in this study because of the similarity in concentrations found in different parts of the body and the use of the entire depth of blubber in the analysis.

# Interpretation of regional differences

There have been few reports in the literature regarding use of chlorinated hydrocarbon concentrations or ratios to examine movements and intermixing in marine mammals. Winn and Scott (1978) included differences in PCB and DDT concentrations as part of the evidence for separate stocks of humpback whales in the western North Atlantic. Gaskin et al. (1982) noted differences in DDT concentrations in harbor porpoise from inside and outside the Bay of Fundy. Calambokidis et al. (1984, 1977) reported differences in the PCB/DDT ratio in Washington harbor seals and its usefulness in evaluating regional movement and interchange. There was minimal or no overlap in the ratio of PCB/DDE in 73 harbor seal blubber samples from some regions in Washington This technique was especially valuable in Washington State because of the extreme differences in contaminant levels and ratios that exists between the protected waters of Puget Sound and the Hood Canal and the Washington outer coast. Harbor seal body burdens of contaminants, intake from prey species, metabolism, and excretion were also evaluated. These studies indicated contaminant concentrations in harbor seal blubber represented the accumulation over prolonged periods with minimal except through lactation or transplacental transfer (Calambokidis et al. 1978).

Gaskin et al. (1983) noted that PCB concentrations were strongly correlated to HCB concentrations in harbor porpoise from the Bay of Fundy, and suggested that the dynamics of uptake and metabolism were likely similar for both compounds. Unfortunately he did not report comparisons to DDT concentrations in that Clausen et al. (1974) reported the lack of a correlation between DDE and PCB in Arctic marine mammals including harbor The lack of correlation suggested to these authors that marine mammals may be able to metabolize different chlorinated hydrocarbons. An alternate hypothesis that explains their data, however, is that the different species tested had differential food habits or movements that would explain the

<span id="page-14-0"></span>Interpretation of the significance of contaminant ratios requires several conditions or assumptions:

Contaminant concentrations are reasonably stable in an individual animal and would change very gradually if an animal moved and was exposed to different contaminants: This is supported by the high contaminant concentrations in harbor porpoise and other marine mammals and the accumulation by age that has been seen. Both indicate contaminants are accumulated over long periods and therefore could not change rapidly.

A gradient in contaminant concentrations and the ratios of different contaminants exists in the environment alonq the species range being examined: If no gradient in the ratio of contaminants exists then they would not serve as useful indicators of possible movement. This appears to be the case in California where a strong gradient exists with PCB and DDT because of the heavy DDT contamination in Southern California. There may not be as strong a gradient along the coast of Oregon, however, making interpretation of within Oregon results more difficult.

Harbor porpoise in different areas don't feed heavily on migratory fish coming from outside the area: Contaminant concentrations and ratios reflect those in their prey. Harbor porpoise feeding on prey that have migrated from outside the locality will not reflect the local contaminant concentrations or ratios. This problem would likely result in a greater variation in results from a given area and suggest movement of animals even where it is not occurring.

Animals sampled include all possible age/sex classes that might be involved in movement: With many species of marine mammals movements and/or dispersals occur differentially between different sexes and age classes.

Not all of the above conditions can yet be met and sample size is limited. The preliminary results from California, however, are consistent with a hypothesis of limited regional interchange between harbor porpoise populations in the three regions of California examined. The strong gradient in contaminant ratios that occurs along the southern and central California coast makes it an ideal area to employ contaminant levels and ratios to examine interchange of animals between regions.

#### CONCLUSIONS

- Contaminant concentrations in harbor porpoise were closely correlated with each other and contaminant ratios were far more consistent in relation to location than any single contaminant concentration.

<span id="page-15-0"></span>- Highly significant patterns in contaminant ratios were found by location both among the three states tested and within California. The use of contaminant ratios for examining geographic interchange of animals appears promising.

- Geographic patterns in contaminant ratios were most pronounced in California and most variable in Oregon.

- Preliminary results are consistent with the hypothesis of limited harbor porpoise interchange between broad geographic areas and in California possibly neighboring areas, though sample size is limited.

- Significant associations were found between contaminant<br>concentrations and year of collection, length, and blubber<br>thickness.

- Position on the body from which blubber samples are collected is not of major importance. Only minimal variations were found in contaminant concentrations or ratios among blubber samples collected from different areas of two harbor porpoise.

#### RECOMMENDATIONS

Sample size remains the most limiting factor in interpretation of the results from California. Initially this contract was intended to include the analysis of 38 samples from California with the balance of samples from Oregon and Washington. Because of difficulties in obtaining samples, only 15 different animals from California were tested with the balance of the samples consisting of samples from Oregon or Washington, or duplicate samples from the same animals. Increasing the sample size of animals from California would be extremely important to test for the preliminary differences that were revealed in this study.

Determination of food habits and testing of stomach contents of some of the California harbor porpoise would be valuable This would determine the degree to which harbor porpoise may be feeding on prey that reflect local contaminant concentrations, information on contaminant concentrations in harbor porpoise prev would also allow calculation of contaminant uptake and accumulation dynamics. *y*

PCB, DDE, and HCB appear to be good choices of contaminants for analysis in future samples. It may be fruitful, however, to experiment with some other contaminants that may also be good indicators. The most important criteria for additional The most important criteria for additional contaminants to test is that: **1**) they be stable and accumulate in harbor porpoise, 2) occur in the blubber, 3) occur in varying amounts in the environment within the geographic region being examined.

#### **ACKNOWLEDGMENTS**

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(-) - Indicates data not available or known.

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Table 3.

(-) - Indicates data not available or known.

 $17$ 

.0620 .0770

 $.0460$ <br> $.0470$ <br> $.0342$ 

 $1.32$ <br> $1.32$ <br> $1.05$ 

 $1.76$ <br> $.840$ <br> $1.78$ 

 $38.3$ <br> $17.7$ <br> $52.1$ 

 $22.9$ <br> $13.5$ <br> $49.6$ 

4180

 $2.0$ <br> $2.0$ 

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883

OSU885-671 Agate Bch.

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<span id="page-23-0"></span>Background information and results of analysis of harbor porpoise blubber samples from Washington. Background data provided by organizations listed in Table 1. Blubber thickness reported by<br>collector or if not available measured on subsamples provided for analysis. Table 4.



(-) - Indicates data not available or known.

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Summary of contaminant concentrations (ppm, wet wt.) and ratios by region and state. Table 5.

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Figure 1. Chromatogram of 5 ul injection of a 1.5 ng/ul PCB standard mix consisting of equal parts of the commercial PCB mixtures Aroclor 1242, 1254, and 1260. Numbers indicate the retention time in minutes from the start of the injection. Column is 6' glass packed with 10% DC-200 on Gas Chrom Q with an alkaline precolumn.

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Figure 2. Chromatogram of a 3 ul injection of the cleaned-up extract of the blubber of MMP-92, a harbor porpoise from Washington State. The peak eluding at 1.99 is HCB and the peak at 6.35 is DDE. All peaks after the DDE peaks are from PCBs. Numbers indicate the retention time in minutes from the start of the injection. Column is 6' glass packed with 10% DC-200 on Gas Chrom Q with an alkaline precolumn.

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Figure 3. Chromatogram of a 9 ul injection of the cleaned-up extract of the blubber of LML 85-6, a harbor porpoise from California. The peak eluding at 1.99 is HCB and the peak at 6.35 is DDE. All peaks after the DDE peaks (except at 8.36) are from PCBs. Numbers indicate the retention time in minutes from the start of the injection. Column is 6' glass packed with 10% DC-200 on Gas Chrom Q with an alkaline precolumn.

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Figure 4. Map of study area showing locations where harbor porpoise were collected. Bars indicate regions clumped for statistical tests reported in text.

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DDE (PPM, WET WT.)

Figure 5. Concentration of DDE in harbor porpoise blubber (ppm, wet wt.) plotted against latitude.

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DDE/PCB RATIO

Ratio of DDE to PCB (DDE/PCB) in harbor porpoise blubber plotted against latitude. Figure 6.

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 $\ddot{\phantom{0}}$ Figure 7. Ratio of HCB to DDE in harbor porpoise blubber plotted against latitude.

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Figure 8. Mean and range of DDE/PCB ratio found in harbor porpoise blubber by region. (\*) - one sample collected in 1971 not included.