Correlates of Cytochrome P450 1A1 Expression in Bottlenose Dolphin (Tursiops truncatus) Integument Biopsies

Joanna Y. Wilson,*† Randall Wells,‡ Alex Aguilar,‡ Asuncion Borrell,‡ Victoria Tornero,‡ Peter Reijnders,§ Michael Moore,* and John J. Stegeman*

*Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543; †Chicago Zoological Society, c/o Center for Marine Mammal and Sea Turtle Research, Mote Marine Laboratory, Sarasota, Florida 34236; ‡Department of Animal Biology, University of Barcelona, Barcelona 08028, Spain; and §Alterra—Marine and Coastal Zone Research, 1790 AD Den Burg, The Netherlands

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Integument biopsy is a nondestructive method for sampling free-ranging cetaceans, which allows for the determination of both contaminant concentrations and biomarker responses. Cytochrome P450 1A1 (CYP1A1) expression is induced by polycyclic aromatic hydrocarbons and planar halogenated aromatic hydrocarbons such as the non-ortho polychlorinated biphenyls (PCBs). CYP1A induction has been used extensively as a biomarker of exposure to such compounds in vertebrates. We measured PCB concentrations and CYP1A1 expression in integument biopsies from bottlenose dolphins (Tursiops truncatus) resident in Sarasota Bay, FL. This population of dolphins has been the subject of long-term population and health assessment, affording the opportunity to evaluate the influence of age, sex, and reproductive status on CYP1A1 expression. CYP1A1 expression was seen in endothelial cells, vascular smooth muscle, and nerve cells in the dermis, similar to what has been observed in other cetacean species. Endothelial CYP1A1 expression varied along the length of the biopsy, which could be related to differences in the structure and functionality of the blubber in different parts of the integument. Neither age nor sex was related to CYP1A1 expression in these biopsies, and reproductive status did not relate to levels of CYP1A1 in females. Total PCB and toxic equivalent quotient concentrations in blubber were positively correlated with dermal endothelial CYP1A1 expression, although mono-ortho PCBs concentrations did not show this relationship. Contaminant concentrations appear to be stronger determinants of CYP1A1 expression in integument of these dolphins, than are age, sex, or reproductive status.

Key Words: cytochrome P450 1A1; CYP1A1; bottlenose dolphin; Tursiops truncatus; biopsy; contaminants.

Determining effects of contaminants in cetacean populations is difficult because experimental exposures are usually precluded for ethical and logistical reasons. Yet, in some bottlenose dolphin populations, exposure to organochlorine contaminants has been related to infectious disease mortality (Kuehl et al., 1994; Lahvis et al., 1995; Lipscomb et al., 1994) and decreased immune function (Lahvis et al., 1995). High-level exposure to organochlorines has also been coincident with cancer (Martineau et al., 2002) and reproductive impairment (Munson et al., 1998; Wells et al., 2005) in cetaceans. As part of the International Whaling Commission’s (IWC) Pollution 2000+ Programme, live bottlenose dolphins (Tursiops truncatus) were sampled to evaluate biomarkers of organochlorine contaminant exposure and effects. The results of a study of cytochrome P450 1A1 (CYP1A1) expression in integument are reported here.

Planar halogenated aromatic hydrocarbons (PHAHs) are known to adversely affect the immune system, development, and reproduction in mammals at low doses (Birnbaum and Tuomisto, 2000). These effects are mediated through binding of the aryl hydrocarbon receptor (AHR), presumably by altering gene expression. The genes whose expression is most highly induced by ligand-activated AHR are those coding for cytochrome P450 1A enzymes (Whitlock, 1999). The induction of CYP1A1 by planar polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and dibenzofurans has been correlated to their toxicity in rodent species (Safe, 1986), although a direct role of CYP1A1 in toxicity of these chemicals has yet to be firmly established (Rifkind, 2006).

Obtaining tissue samples from cetacean species typically has relied on dead stranded animals, native subsistence hunts, or integument biopsies of free-ranging animals. Biopsy offers the best opportunity to sample from healthy animals over a wide range of ages and both sexes (Aguilar and Borrell, 1994a). Blubber collected by biopsy of wild cetaceans has been used for monitoring levels of organochlorine contaminants, including PCBs (e.g., see Gauthier et al., 1997; Ross et al., 2000). Blubber PCB concentrations have been correlated with measures of CYP1A1 in cetacean integument (Fossi et al., 1992; Marsili et al., 1998) and liver (White et al., 1994; Wilson, 2003). CYP1A1 is expressed in several cell types present in...
cetacean integument (Angell et al., 2004), and CYP1A1 in integument was shown to be inducible by in vitro exposure of sperm whale biopsy slices to the prototypical inducer β-naphthoflavone (Godard et al., 2004). Those studies demonstrate that CYP1A1 is present, measurable, and induced by exposure to typical CYP1A inducers in integument of cetaceans. Correlating CYP1A1 expression with chemical exposure in cetacean species could suggest species differences in responsiveness to PHAH and polycyclic aromatic hydrocarbons (PAH) and allow us to prioritize species of cetaceans for toxicological studies. This approach has been successfully used in avian species (Kennedy et al., 1996, 2003) and could represent a model for studies in protected species. Such information could relate to the sensitivity of cetacean species to PHAH toxicity and allow us to infer the likelihood of toxic effects in wild populations in which contaminant concentrations are known.

The Sarasota Bay bottlenose dolphin population provided opportunities to sample individual dolphins for which there is extensive background information on life history (e.g., age, sex, geographic range, and maternity/paternity), reproductive histories, movements in the bay, and health (monitored for five successive generations). Research on this dolphin community resident in Sarasota Bay has been ongoing since 1970, and about 150 identifiable individuals, most with known gender, age, and genetic relationships, are currently monitored (Scott et al., 1990; Wells 2003; Wells and Scott 1990). The Sarasota-based program has been developing methods for assessing the health and population status of these dolphins, not only to monitor the risks to the population but also to assess whether dolphins may serve as sentinels of the health of marine ecosystems as a whole. For this study, integument biopsy samples were collected from known Sarasota Bay dolphins to address the hypothesis that there is a relationship between CYP1A1 expression and the concentrations of AHR agonists, especially PCBs, in the integument. Wells et al. (2005) have examined the relationships between concentrations of organochlorine compounds, including PCBs, and the life history and reproductive success in this population. A representative cross section of the resident dolphin community was sampled, facilitating an analysis of possible relationships between contaminant concentrations, age, and gender with CYP1A1 expression in integument biopsies.

**MATERIALS AND METHODS**

Integument samples (epidermis and underlying dermis or blubber) were collected from free-ranging bottlenose dolphin (*T. truncatus*) in Sarasota Bay, Florida during brief capture-release efforts as part of an ongoing health and population assessment (Wells et al., 2004). Age information was obtained primarily from observations of animals from the time of their birth to previously studied mothers (Wells, 2003), but ages of older animals were determined through examination of growth layer groups in a sectioned and stained tooth (Hohn et al., 1989). Reproductive status including maturity (immature or mature), presence and age of current calf, and parity (number of calves born per female) were provided from current and historical data on this population. Capture-release efforts took place in June of 2000, 2001, and 2002. Biopsies were taken by a veterinarian, with a scalpel and local anesthesia. All of the wedge-shaped biopsies were taken from the same site on the dorsolateral aspect and were the full depth of the integument that includes the epidermis and underlying dermis (i.e., skin, including blubber). From each biopsy, samples of blubber and integument were taken for analyses of PCB concentrations and CYP1A1 expression, respectively. Blubber samples were placed on ice and stored at −20°C, while integument samples were fixed in 10% neutral buffered formalin and stored at room temperature.

**Immunohistochemical analysis of CYP1A1 expression.** CYP1A1 expression was examined using immunohistochemistry, as previously described for marine mammal biopsies (Angell et al., 2004). The monoclonal antibody 1-12-3 to *Stenotomus chrysops* CYP1A was the primary antibody. This antibody detects CYP1A in taxonomically diverse vertebrates including cetaceans (Stegeman and Hahn, 1994) and sees a single band in beluga whale liver microsomes (White et al., 1994), ostensibly CYP1A1; the antibody recognizes an epitope specific to CYP1A1 in other mammals (Drahushuk et al., 1998). Serial sections were labeled with the nonspecific antibody MOPC31 (Sigma, St. Louis, MO). Stained sections were evaluated under light microscopy for stain occurrence (scale of 0–3: 0 = no cells stained, 1 = diffuse staining of few cells, 2 = multifocal staining, and 3 = all cells staining) and stain intensity (scale of 0–5: 0 = no staining, 1 = very mild/very light pink, 2 = mild/light pink, 3 = moderate/dark pink, 4 = strong/red, and 5 = very strong/dark red) in each cell type. CYP1A1 expression was calculated as the product of the stain occurrence and intensity to generate a semiquantitative index (scale of 0–15). A linear relationship between this staining index and CYP1A protein content detected by immunoblot was shown previously for expression in liver and for CYP1A1 induced in cultured cells (Hahn et al., 1993; Woodin et al., 1997). Samples were randomly assigned to an immunohistochemistry staining run, and all slides were coded prior to processing for staining of CYP1A1. The age, sex, reproductive status, and PCB concentrations in the animals were not known in advance of the immunohistochemical analysis so that the determination of CYP1A1 expression was completely blind. Liver samples from β-naphthoflavone-treated scup (*S. chrysops*) and/or field-collected winter flounder (*Pleuronectes americanus*) were included as positive control samples in each immunohistochemical staining series. These control liver samples had known content of CYP1A1 and served to calibrate the expression of CYP1A1 in unknowns, so as to adjust for any interrun variability in staining.

**Analysis of contaminant residues.** Blubber samples weighing about 3 g were ground with anhydrous sodium sulfate and extracted with N-hexane (residue-free quality) in a Soxhlet apparatus for 5 h. Concentrations of 40 ml and 10 ml of the solution obtained were used to determine the quantity of extractable fat per gram of blubber. The remainder was mixed with sulfuric acid for lipid clean up and centrifuged for 5 min to aid separation of the hydrolyzed lipid from the solvent extract.

Chromatographic analysis was carried out on a Hewlett-Packard 5890-II GC, equipped with an electron capture detector at 350°C. A fused silica capillary column (length 60 m, 0.25 mm internal diameter) coated with SPB-1 was used as the stationary phase (0.25 μm film thickness). The splitless technique was used to inject 1 μl of the purified extract. Pure nitrogen at a flow rate of 1 ml min⁻¹ was used as a carrier gas. Temperature was programmed as follows: injection at 40°C for 1 min and increased to 170°C at a rate of 25°C min⁻¹; 1 min constant, to 250°C at a rate of 2°C min⁻¹; and then to 280°C, at 5°C min⁻¹.

A preliminary screening of the samples revealed that heptachlor was not present in the tissues analyzed. Therefore, this compound (0.1 mg kg⁻¹) was used as an internal standard. The samples were analyzed for PCBs. Concentrations were expressed in mg kg⁻¹ lipid weight basis. Blanks of pure N-hexane were run daily to ensure the purity of the system. Recoveries of all of the organochlorine compounds were calculated by adding known quantities of...
standard to 12 homogenate replicates of the same sample; recoveries ranged from 82 to 101%. The laboratory (University of Barcelona, Spain) participated in interlaboratory calibration exercises for organochlorine compounds in biota, organized by Quasimeme (1998) and National Institute of Standards and Technology and National Oceanic and Atmospheric Administration (2000). Data from the laboratory were in good agreement with those for reference materials.

Total PCB (tPCB) concentration was calculated as the sum of 22 congeners resolved as individual peaks (IUPAC numbers 28, 52, 95, 101, 105, 118, 128, 138, 149, 151, 153, 156, 170, 174, 180, 183, 187, 183, 194, 195, 201, 206, and 209). The \( \sum \text{mono-ortho} \) PCB concentration was calculated as the sum of four individual peaks (IUPAC numbers 28, 105, 118, and 156). A toxic equivalent quotient (TEQ) concentration of the \( \sum \text{mono-ortho} \) PCBs, including CB118, CB105, and CB156, was calculated using established toxic equivalency factors (TEFs) (Ahlborg et al., 1994; Van den Berg et al. 1998). A TEF for CB28 was not available for the inclusion of this congener.

Statistical analyses. All statistics were performed using Statistica 5.5 (Statsoft Inc. Tulsa, OK). Differences between males and females, for each CYP1A1 score, and differences between cell types (in both males and females) were examined using a \( \times \) test with \( p < 0.05 \). Individual congener concentrations that were below the detection limit (0.001 mg kg\(^{-1}\)) were set at the detection limit for inclusion in statistical analyses. PCB concentrations were transformed to normalize the data for statistical analyses.

## RESULTS

Integument samples were collected from 59 known bottlenose dolphins, 25 males and 34 females, from the population resident in Sarasota Bay, Florida. Animals were biopsied in the summers of 2000, 2001, and 2002, during temporary capture-release events as part of a long-term population and health assessment study. Blubber samples were taken from 47 animals for determination of contaminant concentrations. Ages were known for 80% of the animals sampled. Males ranged in age from 2 to 43 years and females from 2 to 50 years. The ages of the animals biopsied were evenly distributed across the life span of this species. In males, which reach sexual maturity after 10 years, 52% were from animals below the age of sexual maturity. In females, which reach sexual maturity as young as 5 years, 21% were from animals below the age of sexual maturity. Of the females, 22 were known to be mature (15 of them had calves and seven had previously had calves), 11 were immature, and 1 was of age to reproduce but had yet to have a calf. Ages of calves ranged from 2 to 5 years.

PCB concentrations ranged from 1.86 to 215.4 mg kg\(^{-1}\) in blubber (on a lipid basis), but were considerably higher in males than females (Table 1). Although the range of PCB concentrations in males overlapped those in females, mean PCB concentrations were, approximately, three to five times higher in males, depending on the measure of contaminants (PCBs, \( \sum \text{mono-ortho} \) PCBs, or TEQ). In most animals, the \( \sum \text{mono-ortho} \) PCB concentrations were less than 7 mg kg\(^{-1}\).

CYP1A1 expression was seen in endothelial cells of the arterial system and capillaries, vascular smooth muscle cells, and nerve cells (Fig. 1). The staining attributed to nerve cells may include some staining of fibroblasts, which are difficult to distinguish with this immunohistochemical technique. We have not verified that this staining is restricted to neurons, using a neuron-specific stain. However, fibroblasts elsewhere in the dermis were not seen to express CYP1A1 in this study, nor by Angell et al. (2004). CYP1A1 expression was not seen in integumentary epithelial cells, connective tissue, adipocytes, or the perineurium, the dense connective tissue surrounding nerve bundles. The levels of CYP1A1 expression were not significantly different between males and females for any cell type (data not shown), and thus, the results for males and females were combined for subsequent analyses unless specifically noted. Of the cell types showing CYP1A1 expression, levels were lowest in nerve cells and highest in endothelial cells (Fig. 2). Staining was statistically different between cell types.

Endothelial CYP1A1 staining showed variation within the dermis, varying along the depth of the blubber. Some cetaceans, including bottlenose dolphins, have predominantly connective tissue and few or no adipocytes near the epidermal/dermal interface (Sokolov, 1960), denoted as the subcutaneous layer of the dermis by Sokolov (1960) or upper dermis in this paper. The upper dermis corresponds, in part, to the region often referred to as the outer blubber in many studies of lipid and fatty acid content in cetaceans (e.g., see Koopman et al., 1996, 2002). The region referred to as the lower dermis in this paper corresponds to the region where adipocytes dominate, i.e., the subcutaneous fat tissue according to Sokolov (1960) or,

![TABLE 1](http://toxsci.oxfordjournals.org/)

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<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td>PCBs</td>
<td>65.45 ± 51.68 (13.22–215.45)</td>
<td>13.46 ± 12.34 (1.86–46.13)</td>
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<tr>
<td>( \sum \text{mono-ortho} ) PCBs</td>
<td>4.56 ± 2.95 (1.38–14.53)</td>
<td>1.48 ± 1.57 (0.175–5.75)</td>
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<tr>
<td>TEQ</td>
<td>320.25 ± 192.84 (102.8–927.8)</td>
<td>112.8 ± 119.2 (11.31–455.6)</td>
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<tr>
<td>( N )</td>
<td>21</td>
<td>25</td>
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\( ^{a} \) Sum of 22 PCB congeners, see “Materials and Methods” section for specific congeners measured in this study.

\( ^{b} \) Sum of the mono-ortho PCBs (CB28, CB118, CB105, and CB156).

\( ^{c} \) 2,3,7,8-tetrachlorodibenzo(\( \beta \))dioxin (TCDD) equivalent concentration of mono-ortho PCB congeners CB105, CB118, and CB156. See “Materials and Methods” section for details of contaminant analysis.
more generally, the hypodermis. In the upper dermal region, endothelial CYP1A1 expression was significantly less than in the lower dermis, where adipocytes are the dominant cell type (Fig. 2).

CYP1A1 expression in endothelial cells from the lower dermis was correlated with CYP1A1 expression in both nerve cells ($r = 0.62$, $p < 0.05$) and vascular smooth muscle ($r = 0.57$, $p < 0.05$; Table 2). CYP1A1 expression levels in smooth muscle and nerve cells also were correlated with one another ($r = 0.72$, $p < 0.05$) (Table 2). In contrast, CYP1A1 expression in endothelial cells from the upper dermis was not correlated to CYP1A1 expression in any other cell type (Table 2). Age and sex did not appear to have a strong influence on CYP1A1 expression (Fig. 3), and age was not significantly correlated with CYP1A1 levels in any cell type, regardless of whether sex was used as a grouping factor (data not shown).

CYP1A1 expression in endothelial cells of the upper dermis, vascular smooth muscle, and nerve cells did not correlate with PCB concentration in the blubber, regardless of whether or not sex was used as a grouping factor. However, for the total set of samples, endothelial CYP1A1 expression in the lower dermis was not different in females of different reproductive status (immature, mature with calf, and mature without calf), females with calves of different ages ($\leq 2$ years or $> 2$ years), or with parity (1–5 calves born, data not shown).

CYP1A1 expression in endothelial cells of the upper dermis, vascular smooth muscle, and nerve cells did not correlate with PCB concentration in the blubber, regardless of whether or not sex was used as a grouping factor (data not shown). Furthermore, CYP1A1 expression in endothelial cells of the lower dermis was not different in females of different reproductive status (immature, mature with calf, and mature without calf), females with calves of different ages ($\leq 2$ years or $> 2$ years), or with parity (1–5 calves born, data not shown).

CYP1A1 expression in endothelial cells of the upper dermis, vascular smooth muscle, and nerve cells did not correlate with PCB concentration in the blubber, regardless of whether or not sex was used as a grouping factor. However, for the total set of samples, endothelial CYP1A1 expression in the lower dermis was weakly correlated with tPCB ($r = 0.38$, $p < 0.01$, Fig. 4) and TEQ concentrations ($r = 0.32$, $p < 0.03$) but not with $\sum$mono-ortho PCB concentrations. When animals were grouped by sex, no relationship between endothelial CYP1A1 in the lower dermis and any of the measures of PCB content was found. Multiple regression analysis was performed to determine if an interaction between age, sex, and contaminants could better explain the CYP1A1 expression in the lower dermis. None of the partial coefficients of correlation ($\beta$) were significant for age or sex in these analyses, regardless of which measure of PCB concentration was used.
DISCUSSION

Considering that cetaceans are protected species with long lives and high lipid reserves and are top predators, the impacts of lipophilic contaminants are of concern, yet must be studied with limited access to tissues and controlled experiments. Studies of contaminant responses in the integument currently offer one of our only options to investigate impacts of contaminants in free-ranging populations of cetaceans.

CYP1A1 is expressed in several cell types present in cetacean integument, and this expression varies both within species and between species, possibly reflecting differences in exposure and responsiveness to AHR agonists, most likely PHAH and/or PAH (Angell et al., 2004). The usefulness of measuring CYP1A1 as a biomarker of exposure, and possibly effects, in integument of free-ranging cetaceans will depend on the nature of dose-response relationships, our understanding of the influence of age, sex, and reproductive status, and our understanding of the relationship between expression of CYP1A1 in integument and other internal organs and overall health impacts. While correlation between CYP1A1 expression in integument and other organs of beluga whale was not seen in one study (Wilson et al., 2005), blubber PCB levels were correlated with hepatic CYP1A1 levels (White et al., 1994). However, endothelial cells from different organs of dolphin differ in their response to AHR agonists (Garrick et al., 2006). CYP1A1 expression in skin or integument appears to be less responsive to inducers than in some internal organs in beluga (Wilson et al., 2005) and in harbor seal (Miller et al., 2005). Determining dose-response relationships using cells in culture (Garrick et al., 2006) or tissue slice techniques (Godard et al., 2004) could indicate the degree to which the integument is less responsive than other tissues.

The biopsy of free-ranging animals does not usually involve temporary capture-release and collection of life history data. Moreover, biopsy samples taken from unrestrained animals are inconsistent in sampling site on the body and depth of dermis obtained. The study of CYP1A1 expression in biopsies taken

<table>
<thead>
<tr>
<th>ENDOTHELIAL CELL—UPPER DERMIS</th>
<th>ENDOTHELIAL CELL—LOWER DERMIS</th>
<th>VASCULAR SMOOTH MUSCLE</th>
<th>NERVE CELL</th>
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<tbody>
<tr>
<td>0.22</td>
<td>0.29</td>
<td>0.09</td>
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<tr>
<td>NS</td>
<td>0.57</td>
<td>0.62</td>
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Note. NS means not significant.
*p < 0.05.

FIG. 3. CYP1A1 expression in endothelial cells from the lower dermis related to age and sex in bottlenose dolphin.

FIG. 4. Correlation of tPCBs and CYP1A1 expression in endothelial cells from the lower dermis. Correlations are weakly significant ($r = 0.38, p < 0.01$) and include both males and females. Contaminant data was not available for all animals in this study, so only those samples with both CYP1A1 and PCB data are shown. See “Materials and Methods” section for details.
from the bottlenose dolphin in Sarasota Bay provides a rare opportunity to examine the influence of life history parameters on CYP1A1 expression in a cetacean species; specifically age, sex, and reproductive status. An understanding of the influence of these parameters is important for our ability to apply and interpret CYP1A1 expression in other cetaceans, where life history data are unknown (Angell et al., 2004). Animals were temporarily restrained, and all samples were taken from the same site and included the full depth of the dermis.

**Cell Types that Express CYP1A1 in Bottlenose Dolphin Integument**

The detection of CYP1A1 expression in vascular endothelial cells, vascular smooth muscle, and nerve cells but not in epidermal cells, adipocytes, and connective tissues is similar to prior results obtained with this and other cetacean species (Angell et al., 2004). In agreement with the findings in other cetaceans (Angell et al., 2004), the predominant cell type expressing CYP1A1 in bottlenose dolphin integument was the vascular endothelium.

Endothelial CYP1A1 expression varied between the layers of the dermis. The upper dermis, an area characterized by connective tissue and few adipocytes, had lower levels of CYP1A1 expression than in the lower dermis, an area that is predominately composed of adipocytes. Common and bottlenose dolphins show similar differences in vascular endothelial CYP1A1 along the depth of a biopsy sample (Wilson, 2003), but this has not been seen in all cetacean species examined (Angell, personal communication). Certainly, the depth of the biopsy may be an issue in CYP1A1-staining variability in some species examined to date, particularly large whales, where biopsy samples typically include only a portion of the dermis (Angell, personal communication).

The reasons for differences in CYP1A1 expression in different parts of the dermis are unknown; differences in regional blood flow/perfusion and/or lipid dynamics may be involved. Interestingly, studies on lipid content and fatty acid analyses show that the outer blubber is mostly structural and has a thermoregulatory function, while the inner blubber is mostly a site for fatty energy stores (Koopman et al., 1996, 2002). The inner and outer blubber regions overlap but do not directly correspond to the regions showing lower and higher levels of endothelial CYP1A1 expression, respectively. Still, the general correspondence of these regions suggests that lipid content, fatty acid content, and lipid dynamics may be important factors for endothelial CYP1A1 expression in cetacean integument.

The nutrition and reproductive status of an animal may have a major influence on lipid dynamics and the expression of CYP1A1 in endothelial cells of the lower dermis, particularly for females because of the energy demands of pregnancy and lactation. A recent study of CYP1A1 along the full depth of blubber from bottlenose dolphin taken off the Southeast United States supports the idea that lipid dynamics and the reproductive status of females may be important factors in the expression of CYP1A1 in the lower dermis (Montie, 2006). Yet, in our study, measures of reproductive status were not indicative of CYP1A1 expression. The calves in this study were at least 2 years old, the age at which calves are considered nutritionally independent (Wells and Scott, 1999), although lactation continues throughout mother-calf association in this species (Wells and Scott, 1999; Wells et al., 2005).

Whether contaminant concentrations vary within the dermis of cetaceans is not yet clear. When expressed on a lipid basis, contaminant concentrations were not found to vary along sections of blubber of minke and blue whales (Gauthier et al., 1997), but such variation, with higher levels in inner blubber, was observed in fin and sei whales (Aguilar and Borrell, 1991). Further studies are needed to establish whether differences in contaminant concentrations in different parts of the blubber could account for the regional differences in CYP1A1 expression.

**Influence of Life History Parameters on CYP1A1 Expression**

Persistent chemicals such as PCBs accumulate over time and tend to increase with age in male cetaceans, while in reproductively active females these same chemicals typically decrease with age, as a consequence of elimination during pregnancy and lactation (Aguilar and Borrell, 1994b). PCB concentrations were significantly positively correlated with age in males and not correlated with age in females in this study, as expected for small cetaceans without significant reproductive senescence (see Wells et al., 2005 for a discussion of this relationship). In our study, CYP1A1 expression was not directly related to age (Fig. 3), mean CYP1A1 expression was not different between males and females in any cell type, and age and sex were not significant factors in a multiple regression including CYP1A1 and PCB concentrations. In Arctic beluga and in white-sided dolphin, CYP1A1 expression in liver was correlated with Σmono-ortho PCBs in blubber, and this correlation was not dependent on sex (White et al., 1994; Wilson, 2003), as data for both males and females fell on the same regression curves. However, the effect of age was not controlled for in those studies. The data from those and from the present study suggest that age and sex alone may not be important factors in CYP1A1 expression in some cetaceans. Establishing whether this is common among cetaceans will be important for the interpretation of biopsy data.

**Influence of Contaminants on CYP1A1 Expression**

Both PAHs and PHAHs, including planar PCBs, could be expected to be inducers of CYP1A1 in cetaceans. There could be dietary and respiratory exposure to PAH inducers, which could be an important part of the CYP1A induction observed here. Sarasota Bay has inputs from an urban/suburban watershed and is heavily trafficked by hydrocarbon-burning powerboats (Nowacek et al., 2001), a source for such PAHs. Animals
from this population have been described with anthracosis in the mediastinal lymph nodes, a gross pathological indication of substantial PAH exposure in as much as the carbon particles observed in this condition would have been derived from aerial soot (Rawson et al., 1991). However, PAH concentrations were not measured in this study, and thus, their contribution to CYP1A1 expression remains to be addressed.

CYP1A1 expression might be expected to show correlation with PCB concentrations in these dolphins, as PCBs are common and persistent in the environment. In Arctic beluga and white-sided dolphin, CYP1A1 expression in liver was strongly related to Σmono-ortho PCBs in blubber (White et al., 1994; Wilson, 2003). CYP1A1 was induced in a dose-dependent manner in sperm whale biopsy slices exposed in vitro to β-naphthoflavone (Godard et al., 2004). These studies together indicate that levels of CYP1A1 expression and the concentration of CYP1A1 inducers could be related in cetacean integument. Studies of the relationship between contaminant concentrations and benzo(a)pyrene monooxygenase activity, which is catalyzed by CYP1A1, in the skin of cetaceans from the Mediterranean Sea have shown weak relationships with dichlorodiphenyltrichloroethanes and PCBs ($r_2 < 0.52$, Fossi et al., 1992; Marsili et al., 1998). In the present study, CYP1A1 expression in endothelial cells of the lower dermis was weakly correlated to tPCB and TEQ concentrations but not to Σmono-ortho PCBs. Similar results have been obtained in bottlenose dolphin from another location (Montie, 2006). In the present study, the Σmono-ortho PCB concentrations were typically lower than 7 mg kg$^{-1}$ of blubber and the non-ortho PCBs, which are stronger inducers of CYP1A1 expression, were not measured. Defining relationships between tPCBs or subsets of congeners and CYP1A1 expression as a marker may help to determine whether there are adverse health outcomes associated with the low-level exposure to PCBs, an unresolved issue in cetaceans as well as other organisms.

Comparisons of CYP1A1 Expression between Cetacean Species

The levels of CYP1A1 expression seen in Sarasota bottlenose dolphin are similar to those reported in other coastal populations of this species (from off San Diego and the Gulf of Mexico) but higher than those reported for bottlenose dolphin from the Mediterranean Sea and offshore in the Western Atlantic Ocean (Angell et al., 2004). However, contaminant concentrations were not reported for any of those previous samples. When compared to populations with known contaminant concentrations, CYP1A1 levels in integument suggest differences in sensitivity between species. The Arctic beluga expresses similar levels of CYP1A1 (Wilson et al., 2005) as the Sarasota bottlenose dolphins, even though blubber PCB concentrations in the dolphin on average are 20 times higher than those in the Arctic beluga (Muir et al., 1996). *Orca* from the Pacific Northwest have some of the highest PCB concentrations recorded for cetaceans (Ross et al., 2000), and yet, CYP1A1 levels detected in *Orca* integument are among the lowest seen to date (Angell et al., 2004). It is possible that *Orca* are relatively unresponsive, compared to beluga, although it also is possible that there may be differences in cross-reactivity with Mab 1-12-3, which was used with most species examined to date. It will require a concurrent measurement of contaminants and CYP1A1 expression in all of these species, and to evaluate antibody cross-reactivity, to substantiate these interpretations.

CONCLUSIONS

The potential usefulness of the CYP1A1 expression in integument as a biomarker of exposure to AHR agonists in cetaceans is supported by our results. As with CYP1A1 expression in cetacean liver (White et al., 1994; Wilson, 2003) and skin (Fossi et al., 1992; Marsili et al., 1998), the endothelial CYP1A1 expression in integument is correlated, albeit weakly, with PCB concentrations in blubber. That this relationship has been found in different tissue types and with different species is an indication that CYP1A1 expression may be widely applicable as a biomarker of exposure in cetaceans. Better understanding of the role of lipid dynamics, lipid and fatty acid content, and nutritional and reproductive status is needed to ascertain the controlling factors of CYP1A1 expression in integument biopsy samples. Our results suggest that the reproductive status and the life history parameters of age and sex alone do not have a strong influence on CYP1A1 expression in the Sarasota dolphin population. Determining whether this applies to other cetaceans would indicate application of this technique to free-ranging cetaceans, for which life history data are usually lacking. Observations that hepatic CYP1A1 levels relate to blubber PCB levels (e.g., White et al., 1994) indicate that induction of CYP1A1 in integument also may signal related levels of induction in internal organs. Dose-response relationships for CYP1A1 induction in integument endothelial cells as compared to other organs and cell types (e.g., Garrick et al., 2006) will help further validate the use of this biomarker. Comparing CYP1A1 expression in relation to other inducible genes (e.g., CYP1B1) and/or to results of expression profiling, in populations with or without known pathologies or diseases potentially related to AHR agonists, will indicate the value of CYP1A1 as a possible harbinger of toxic effects of these chemicals in marine mammals.

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