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USING CYTOCHROME P4501A1 EXPRESSION IN LIVER AND BLUBBER TO UNDERSTAND EFFECTS OF PERSISTENT ORGANIC POLLUTANT EXPOSURE IN STRANDED PACIFIC ISLAND CETACEANS

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Abstract: Elevated levels of persistent organic pollutants (POPs) have been reported in tropical Pacific Island cetaceans and their environment. In addition, recent health concerns in cetacean populations have warranted investigation into potential physiological effects from POP exposure for this region. Cytochrome P450 1A1 (CYP1A1) is a candidate for examining such effects. This well-studied biomarker of exposure and effect was examined in stranded cetacean liver using immunoblot ($n = 39$, 16 species) and blubber using immunohistochemistry ($n = 23$, 10 species). Paired tissue samples allowed for CYP1A1 comparisons not only between species but also within each individual animal to examine differences between tissue types. Liver CYP1A1 expression correlated positively and significantly with blubber concentrations of all POP categories ($n = 39$, $p < 0.050$) except octachlorostyrene and pentachlorobenzene ($p > 0.100$). Among *Stenella* species, liver CYP1A1 tissue expression was correlated negatively with the sum of all blubber layer endothelial cell CYP1A1 expression ($n = 14$, $p = 0.049$). Overall, elevated expression of liver CYP1A1 confirms its use as a biomarker of POP exposure to cetaceans stranded in the tropical Pacific basin. *Environ Toxicol Chem* 2015;34:1989–1995. © 2015 SETAC

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INTRODUCTION

Recent reports of high levels of persistent organic pollutants (POPs) in marine mammals in the remote tropical Pacific Islands region [1–3] reaffirm the global distribution and persistence of these contaminants. Persistent organic pollutants are known to affect cetacean health by compromising immune system function, decreasing reproductive success, disrupting stress responses, and developmental toxicity [4–7]. Because reported POP levels in Hawaiian marine mammals exceed presumed thresholds for negative health effects [1,8–10], a closer examination of specific physiological impacts in relatively understudied and diverse cetacean species in Hawaii and the tropical Pacific region is warranted.

Cytochrome P450 (CYP) monooxygenases belong to a superfamily of enzymes that play roles in metabolizing toxicants [11]. One extensively studied CYP pathway is the aryl hydrocarbon receptor (AhR) pathway, in which CYP1A1 (CYP family 1, subfamily A, polypeptide1) protein induction occurs after exposure to AhR agonists [12]. Typical AhR agonists are environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs) and some POPs, including polyhalogenated dioxins, furans, and planar polychlorinated biphenyls (PCBs). The induction of CYP1A1 through the AhR pathway has been well documented as a biomarker for exposure to PAHs and certain POPs in a variety of animal species [13] and reflects the response of an organism to contaminants within hours of exposure [14].

Adaptively, CYP1A1 enzymes metabolize PAHs through oxidation and lead to eventual elimination of the compound through excretion. This pathway can also lead to elimination of certain POPs as well. On the other hand, CYP1A1 oxidation of PAHs or POPs has risky trade-offs, because this reaction can produce toxic metabolites or byproducts. For example, some bioactivated CYP1A1 products have the potential to react with critical cell components prior to phase II metabolism. For this reason, some PAHs are well-known mutagens and carcinogens. Certain dioxins and PCBs have planar structures similar to PAHs that allow a close but imperfect fit into the active site of the enzyme, producing an uncoupling of electron transfer during the oxidation reaction that can result in the release of reactive oxygen species into the cell [15].

The formation of reactive intermediates of PAH or certain POPs prior to excretion is yet another toxic pathway response (i.e., hydroxylated (OH) PCBs, methyl sulfone ($MeSO_2$) PCBs, and benzo[*a*]pyrene epoxides) [16,17]. Such intermediate metabolites may result in endocrine disrupting effects via interference with thyroid hormone homeostasis, as found in polar bears (*Ursus maritimus*) [18]. In killer whales (*Orcinus orca*) from the Northeast Pacific, thyroid hormone α receptor (TRα) messenger RNA levels were found to be elevated in individuals with higher levels of PCBs [19]. This was consistent with another study on harbor seals (*Phoca vitulina*) from British Columbia, Canada, and Washington, USA, in which elevated TRα messenger RNA levels were hypothesized to reflect a contaminant-related increase in metabolic turnover [20].

Several lines of evidence have shown that the CYP1A1 pathway can serve as a biomarker of POP exposure in cetaceans. In the beluga whale (*Delphinapterus leucas*), liver CYP1A1 was induced in whales with higher exposure to dioxin-like, planar PCBs [21]. Certain dioxins and PCBs were shown in vitro to bind specifically and in a structure-dependent manner consistent with other well characterized mouse and human AhRs [22,23]. Significant positive correlations between CYP1A1 in blubber and blood concentrations of select

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non-ortho and mono-ortho PCBs (measured as toxic equivalents) have been observed in bottlenose dolphins (*Tursiops truncatus*) [24].

For these reasons, we investigated CYP1A1 protein as a biomarker in liver and blubber tissue from cetaceans stranded in the tropical Pacific from the years 1997 through 2011, and related these responses to previously measured POP concentrations in blubber tissues from the same animals [1]. Furthermore, relative CYP1A1 expression measured in liver was compared with CYP1A1 measured in blubber tissues in a subset of the individuals to examine enzyme patterns between tissues.

MATERIALS AND METHODS

Liver and blubber tissue sample collection

Liver tissue samples ($n = 39$) and 23 blubber tissue samples were obtained from Hawaii Pacific University's marine mammal stranding program tissue archive (National Oceanic and Atmospheric Administration [NOAA] permit #932-1905). These samples originated from the same cetacean strandings that occurred in the Pacific Islands regions as previously described [1]. The tissue samples selected for analysis were limited to those from stranded cetaceans considered either code 1 (alive then euthanized) or code 2 (freshly dead) to lessen impacts from tissue degradation [25]. Liver and blubber tissues were subsampled at time of necropsy. Liver tissue was collected in microcentrifuge tubes and stored at −80 °C until analysis. Blubber tissues for histology were stored in formalin. Samples represent up to 16 cetacean species stranded in the Pacific Islands over a 15-yr period (1997–2011) consisting of a mixture of age classes and sexes (individual animals are described in Bachman et al. [1]).

CYP1A1 in liver

Liver tissue prep and gel electrophoresis. Liver tissue samples were prepared for gel electrophoresis using NE-PER nuclear and cytoplasmic extraction kit (Pierce). Tissue samples (<0.025 g) were washed with phosphate buffered saline and then centrifuged at 4 °C (500 rcf) for 5 min. Protease inhibitors were added, tissues were manually homogenized on ice with a glass homogenizer, and a final centrifugation step occurred at 4 °C (16 000 rcf) for 5 min. Colorimetric protein assays were performed to measure total protein in stranded cetacean cytosolic fractions. The unknown cetacean protein samples were plotted against nine known concentrations of bovine serum albumin (BSA) following absorbance measurements with a spectrophotometer at 562 nm (Bio-Rad SmartSpec Plus). The BSA standard curve (protein concentration μg/mL vs absorbance, $R^2 > 0.994$) was created alongside 25% diluted cetacean samples. Cytosolic fractions for each cetacean sample were loaded into a polyacrylamide precast gradient 4% to 20% Tris-Glycine gel (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). Samples were combined with lane marker (Tris hydrochloride, sodium dodecyl sulfate, glycerol, dithiothreitol, and proprietary pink tracking dye) to visualize protein bands and ensure effective transfer. Samples were diluted accordingly with cytoplasmic extraction reagent I solution (Pierce) to ensure uniform protein concentration (50 μg/well) and sample volumes across each well on every gel. Gels were run at a constant 200 volts for 1 h or until lane marker reached the bottom of the gel (Hoefer miniVE electrophoresis unit).

Immunoblotting. Separated proteins in the gel were immediately transferred to nitrocellulose membranes and verified with Ponceau S stain [26]. Each transfer was run at a constant 60 volts for 2 h in an ice bath. After blocking for 1 h with shaking at room temperature (filtered tris-buffered saline with 0.05% Tween-20 (TBST) in 2.5% nonfat milk), membranes were probed with anti-CYP1A1 antibody (Millipore rabbit polyclonal, 1:750 in TBST) or anti-α-tubulin antibody (Novus \Biologicals mouse monoclonal, 1:5000 in TBST) overnight (12–14 h) at 4 °C. The blots were then incubated with a secondary goat anti-rabbit or anti-mouse antibody (Pierce; 1:1000 in TBST) for 2 h shaking at room temperature. A series of TBST rinsing steps were performed between each membrane incubation step to remove nonspecific binding. Chloronaphthol and diaminobenzidine-based (CN/DAB) detection substrate (Pierce) was used for colorimetric visualization. Photographs were taken following 20 min of substrate development with a Canon camera (PowerShot S90) in the laboratory under natural sunlight on a low-light setting.

Quality assurance and quantification of CYP1A1, relative expression in liver. Both positive and negative controls were used on every immunoblot. The positive control was a known cetacean sample with high CYP1A1 expression (sample ID 12470) in replicates of 3, and the negative control was a blank lane with only lane marker to correct for background staining (Figure 1).

The CYP1A1 relative expression in liver tissue was determined with Image J software (National Institutes of Health [NIH]). Photographs of blots were converted to 8-bit TIFF images for analysis. Area measurements or optical density of each protein band accounted for both thickness and intensity relative to the bands total size and density. An area measurement for each blank lane (1 per gel) was subtracted from sample areas for each gel to adjust values for potential background interference. A molecular size marker lane was included on each gel to verify the size of the visualized protein bands.

The CYP1A1 quantification methods were similar to Fossi et al. [27]. The internal standard (sample ID 12470 [striped dolphin] in replicates of 3), blanks and molecular size marker were included on each gel. A calibration curve was created from a diluted series of purified liver microsomes from rats treated with β-naphthoflavone with known CYP1A1 concentrations (R1083; Xenotech). To quantify unknown CYP1A1 relative expression in stranded cetacean samples, the ratio of the amount of known total CYP1A1 protein (pmol) from the standard rat microsomes (R1083) to the amount of CYP1A1 protein (pmol) in the internal standard (sample 12470) was graphed on the

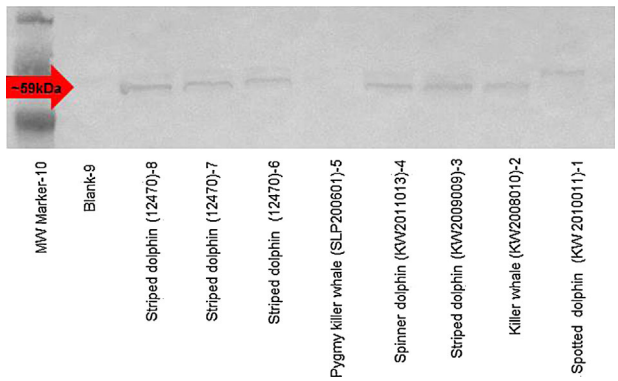

Figure 1. Representative immunoblot showing cytochrome P450 1A1 (CYP1A1) expression in liver tissue of stranded cetaceans from the Pacific Islands. Total protein (50 μg) was loaded in lanes 1 through 5 and lane 8. Total protein (20 μg) was loaded in lane 6, and 30 μg total protein was loaded in lane 7. Internal standard animal is located in lanes 6 through 8. The negative control, or blank used to correct for background staining, is in lane 9.

x-axis. On the *y*-axis, the ratio of optical density was plotted (optical density of R1083:optical density of 12470, $R^2 = 0.998$). The amount of CYP1A1 protein in the internal standard was determined by using the slope and *y*-intercept from a 4-point calibration curve of known standard rat microsomes (pmol of R1083 vs optical density of R1083). This calibration curve bracketed the unknown cetacean samples with detectable CYP1A1 expression. To account for samples that did not show visible CYP1A1 expression, a reporting limit was calculated as the amount of the lowest detectable calibration point (pmol) divided by total protein loaded (mg). The CYP1A1 bands that were not visible were considered below the sensitivity threshold of the immunoblotting method and were reported as <0.399 pmol/mg. To verify that tissue degradation was not a factor in the analyses [26], α-tubulin expression was verified for all 39 stranded cetacean liver tissue samples.

Contaminants in blubber tissue

A suite of POPs was quantified from frozen blubber tissues of paired stranded cetacean samples as previously described [1]. Compounds used for biomarker comparisons are reported in ng/g lipid and include the following summed concentrations: ∑PCBs ($n = 80$), polybrominated diphenyl ethers (∑PBDEs; $n = 28$), dichlorodiphenyl-dichloroethane related pesticides (∑DDTs; $n = 6$), chlordane-related compounds (∑CHLs; $n = 6$), α-,β-,γ-hexabromocyclododecanes (∑HBCDs), α-, β-,γ--hexachlorocyclohexanes (∑HCHs), 4 parlar compounds (∑TOXs), mirex, hexachlorobenzene, octachlorostyrene (OCS), and pentachlorobenzene (PeCB).

CYP1A1 in blubber

Preparation of blubber for histology. Blubber samples for which POP concentrations were known ($n = 23$) were retrieved from formalin storage and placed in ethanol for transport to the Department of Pathobiology, College of Veterinary Medicine at University of Tennessee (Knoxville, TN, USA) or the histology core facility at John A. Burns School of Medicine, University of Hawaii (Honolulu, HI, USA). Blubber samples were trimmed and embedded in paraffin blocks. The embedded tissues were sectioned at 5 μm and placed on Leica Biosystems 1-mm slides. The slides were then returned to Hawaii Pacific University for staining and analysis. Sequential cuts were used for hematoxylin and eosin staining, positive antibody, and negative control slides.

Hematoxylin and eosin staining. Slides were deparaffinized then stained with hematoxylin and eosin (Sigma Aldrich). The slides were mounted with Permount (Fisher Scientific) and dried overnight. Blubber layers (deep, middle, and superficial) were determined based on gradients of adipocyte area, and adipocyte and endothelial cell quantity throughout the blubber layers (K.M. Foltz, 2012, Master's thesis, Hawaii Pacific University, Kaneohe, HI, USA). To determine adipocyte area, a 100-μm scale at 400× total magnification was used to calibrate the measured pixels to distance in Image J software (NIH).

Immunohistochemistry. Slides were first deparaffinized with several changes of xylene and then rehydrated with decreasing concentrations of ethanol and Millipore purified water (18.2 MΩ cm^{-1}) incubations in preparation for immunohistochemical staining procedure. Test slides were treated with anti-CYP1A1 antibody (Millipore rabbit polyclonal, 1:4500 in 0.9% phosphate buffered saline) for 2 consecutive 1-h incubations, each separated by rinse procedures with 0.9% phosphate buffered saline. All subsequent staining steps were performed per manufacturer instructions (Vectastain ABC-AP kit, rabbit IgG).

Quality assurance and quantification of CYP1A1, relative expression in blubber. Because previous studies have shown that the vascular endothelial cells of the arterial system and capillaries are known to express the highest levels of CYP1A1 in the blubber [24], the present study examined CYP1A1 expression in blood vessels lined with endothelium. Intensity and occurrence of CYP1A1 in blood vessels within the blubber layers were determined through use of the Olympus BX43 microscope at a total magnification of 200× (20× objective, 10× eyepiece). Intensity was measured on a scale of 0 to 5, with 0 indicating no staining and 5 indicating the brightest amount of staining as seen in previous studies [14,24,28]. The CYP1A1 expression in each blubber layer was determined as the product of intensity and occurrence divided by the total amount of blood vessels quantified within the respective blubber layer in the hematoxylin and eosin stained slide. This method of determining expression of CYP1A1 within the blubber normalizes expression by the potential vessels quantified that could express CYP1A1.

Each individual test slide was paired with a sequentially cut slide used as a negative control to account for background staining not indicative of CYP1A1 expression. These were processed alongside the test slide, but without the anti-CYP1A1 antibody. Any nonspecific staining was accounted for by subtracting staining quantified within the negative control slides from staining quantified in test slides. For a positive control, each staining run included slides containing tissues known to express CYP1A1 (S. Fertall, 2010, Master's thesis, Hawaii Pacific University, Kaneohe, HI, USA). Slides with no observable CYP1A1 expression were considered below the sensitivity threshold of the immunohistochemistry method and were reported as <0.010 expression/EC, or less than the minimum relative expression detected (reporting limit).

Statistical analysis

The open-source statistics program R NADA package [29] was used for all statistical tests as recommended for left censored data (less than reporting limit) [30]. Mean, median, and standard deviations for blubber POP concentrations and liver CYP1A1 expression were generated through Kaplan-Meier or regression on order statistical models. The POP concentrations and CYP1A1 expression (blubber and liver) were not normally distributed using Shapiro-Wilk goodness of fit test ($n = 39$, $n = 23$, $n = 14$). In addition, residuals from most correlations were not normally distributed; therefore, nonparametric Kendall's Tau correlations were performed.

RESULTS AND DISCUSSION

CYP1A1 expression in liver

Relative CYP1A1 expression in livers of individual stranded cetaceans is shown in Table 1, with a representative immunoblot in Figure 1. Highest CYP1A1 expression occurred in striped dolphin (*Stenella coeruleoalba*), bottlenose dolphin, and spinner dolphin (*Stenella longirostris*) livers. The majority of individuals in the present study expressed what can be considered low to moderate levels of CYP1A1.

In general, liver CYP1A1 expression increased with exposure to POP concentrations in blubber. The CYP1A1 expression correlated positively and significantly ($p < 0.05$) with summed concentration totals of all POP categories, except OCS and PeCB (Table 2). Figure 2 shows the correlation between ∑PCB compounds and CYP1A1 expression in liver.

Table 1. Summary of liver cytochrome P450 1A1 protein expression for stranded cetaceans from the tropical Pacific (1997–2011, 16 species)

		Liver CYP1A1 expression (pmol/mg)	
Common name	n	Median (range)	Mean ± SD
Striped dolphin	6	20.1 (11.8–72.8)	30.7 ± 23.9
Bottlenose dolphin	2	(20.5–23.3)	
Spinner dolphin	10	11.9 (6.24–36.5)	14.1 ± 9.15
False killer whale	1	9.88	
Spotted dolphin	3	9.12 (3.04–14.6)	8.92 ± 5.78
Pygmy killer whale	2	(<0.399–7.23)	
Melon-headed whale	3	7.22 (<0.399–7.22)	7.22 ± 0.00
Killer whale	1	6.89	
Sperm whale	1	3.47	
Humpback whale	3	3.00 (<0.399–3.00)	3.00 ± 0.00
Rough-toothed dolphin	1	2.66	
Blainville's beaked whale	1	2.03	
Cuvier's beaked whale	2	(<0.399–1.99)	
Longman's beaked whale	1	<0.399	
Pygmy sperm whale	1	<0.399	
Dwarf sperm whale	1	<0.399	
All samples	39	7.22 (<0.399–72.8)	11.7 ± 14.1

CYP1A1 = cytochrome P450 1A1; SD = standard deviation.

This is similar to other reports [21,31], and reflects the known mechanistic relationship between liver CYP1A1 and planar PCBs. However, we also investigated relationships between liver CYP1A1 expression and POP compound classes other than PCBs. These organic toxicants are broad acting and their responses in the cell are poorly understood [32]. Interestingly, the majority of POP classes were positively correlated with CYP1A1 expression, and liver CYP1A1 expression correlated strongly with ∑TOXs, ∑PBDEs, and ∑HCHs in blubber ($p < 0.005$). This is most likely because these lipophilic compound classes share chemical properties and co-vary with the subset of POPs that are AhR agonists, not an ability for all POPs to activate the AhR pathway.

Because there are a few reports that suggest that total contaminant burdens can be high enough to suppress CYP1A1 expression, we specifically looked for evidence for suppression of CYP in animals with the highest POPs concentrations. Beluga whales in the St. Lawrence estuary with very high contaminant concentrations in blubber tissue had very low expression of CYP1A1 in liver, in contrast to high CYP1A1 liver expression in Arctic belugas with moderate contaminant burdens [33]. These investigators suggested that very large levels of contaminant exposure (29 600–78 900 ng/g lipid) could potentially suppress liver function and thus CYP1A1 protein expression. In this context, the positive correlations occurring between the majority of POPs in blubber and liver CYP1A1 tissue expression (Figure 2, Table 2) indicate that, in general, stranded cetaceans from this Pacific Islands region (40.8–93 200 ng/g lipid, [1]) are not experiencing metabolic suppression as was indicated by the St. Lawrence beluga whale population and that, in general, CYP1A1 expression in the liver can indicate exposure to POPs in stranded cetaceans in the tropical Pacific.

Table 2. Positive correlations between liver cytochrome P450 1A1 expression (pmol/mg) and all persistent organic pollutants concentrations (ng/g lipid) analyzed in blubber of Pacific Island stranded cetaceans ($n = 39$, 16 species)

Compounds	p value[a]	Tau[a]
∑DDTs	0.028*	0.244
∑PCBs	0.032*	0.239
∑CHLs	0.019*	0.260
∑TOXs	0.005*	0.312
∑PBDEs	0.002*	0.350
Mirex	0.022*	0.254
∑HCHs	0.003*	0.327
∑HBCDs	0.011*	0.283
HCB	0.029*	0.242
OCS	>0.100	0.067
PeCB[b]	>0.100	0.020

[a]Results from nonparametric Kendall's Tau test using R NADA [29].
[b]More than 80% of the samples were below the reporting limit for this contaminant class.
*$p < 0.033$.
∑DDTs = 6 dichlorodiphenyl-dichloroethane related pesticides; ∑PCBs = 80 polychlorinated biphenyls; ∑CHLs = 6 chlordane-related compounds; ∑TOXs = 4 parlar compounds; ∑PBDEs = 28 polybrominated diphenyl ethers; ∑HCHs = α-,β-,γ-hexachlorocyclohexanes; ∑HBCDs = α-,β-,γ-hexabromocyclododecanes; HCB = hexachlorobenzene; OCS = octachlorostyrene; PeCB = pentachlorobenzene.

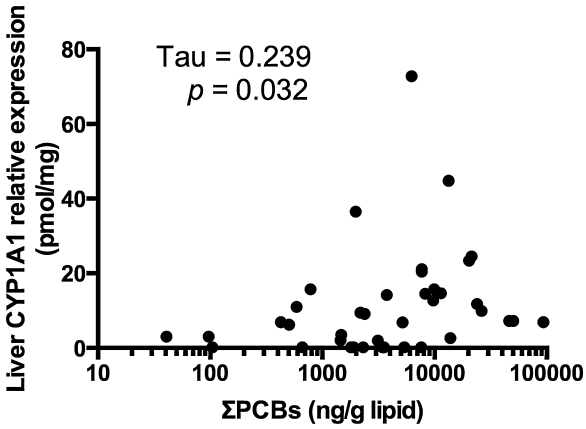

Figure 2. Positive correlation between liver cytochrome P450 1A1 (CYP1A1) expression and blubber summed polychlorinated biphenyls (∑PCB) concentrations of Pacific Island stranded cetaceans ($n = 39$, 16 species). The correlation coefficient (Kendall's Tau) and p value were generated using the NADA package in R [29].

CYP1A1 expression comparison between blubber and liver tissues

To explore CYP1A1 expression patterns in blubber tissue, CYP1A1 expression was measured using immunohistochemistry in 23 of the 39 stranded individuals that represented 10 cetacean species (Table 3). The deep blubber layer of these Hawaiian cetaceans samples had significantly more endothelial cells compared with the superficial and middle blubber layers (K.M. Foltz, 2012, Master's thesis, Hawaii Pacific University, Kaneohe, HI, USA), and endothelial cells of the blubber are known to display the most frequent and strongest CYP1A1 expression compared with other cell types [12]. In the present study, higher CYP1A1 expression (normalized per endothelial cell) was observed in the deep blubber layer (Table 3) compared with middle and outer layers. This is consistent with other studies that indicate that this layer is active in lipid and contaminant mobilization [24,28]. Despite this possible mechanistic link, only ∑HCH concentrations correlated negatively with CYP1A1 expression in the deep blubber layer (Kendall's Tau = −0.296, $p = 0.049$). No significant correlations were found when comparing blubber POP concentrations with blubber CYP1A1 expression either in the sum of all layers or in the deep layer ($p > 0.100$, $n = 23$; Supplemental Data, Table S1).

Table 3. Cytochrome P450 1A1 expression in blubber and liver of individual stranded cetaceans from the Main Hawaiian Islands ($n = 23$, 10 species)

					Blubber (CYP1A1 expression/EC)				Liver (pmol/mg)
Common name	Sample ID	Condition	TEO (% lipid)	Blubber thickness dorsal (cm)	Deep	Middle	Superficial	Sum all layers	CYP1A1 expression
Blainville's beaked whale	KW2010012	NA	76.8	4.60	1.35	<0.010	<0.010	1.35	2.03
Bottlenose dolphin	KW2011001	Robust	33.7	1.10	0.370	0.080	0.070	0.520	20.5
Bottlenose dolphin	KW2011007	Robust	17.9	1.80	0.770	1.69	0.370	2.83	23.3
Cuvier's beaked whale	KW2008008	NA	82.1	NA	<0.010	<0.010	<0.010	<0.010	<0.399
False killer whale	KW2010019	NA	46.5	NA	0.760	0.450	0.170	1.38	9.88
Killer whale	KW2008010	NA	38.8	NA	0.890	2.06	1.16	4.11	6.89
Pygmy killer whale	SLP200601	NA	43.8	2.00	1.54	0.530	0.550	2.62	<0.399
Pygmy killer whale	KW2009006	NA	24.9	2.30	0.070	0.550	1.09	1.71	7.23
Sperm whale	KW2011008	NA	39.9	3.10	<0.010	<0.010	<0.010	<0.010	3.47
Spotted dolphin	KW2008005	Robust	54.5	NA	0.250	0.230	0.090	0.570	9.12
Spotted dolphin	KW2009015	Thin	21.0	0.800	1.60	0.110	0.210	1.92	14.6
Striped dolphin	12470	Robust	28.4	NA	<0.010	<0.010	<0.010	<0.010	24.5
Striped dolphin	KW2008006	Thin	41.1	0.600	0.060	0.020	0.030	0.110	72.8
Striped dolphin	KW2009008	Robust	34.2	1.50	0.090	0.030	<0.010	0.120	15.7
Striped dolphin	KW2009009	Thin	16.4	0.060	0.010	0.090	0.090	0.190	11.8
Striped dolphin	KW2009011	Thin	33.9	0.040	<0.010	0.030	0.230	0.260	44.8
Striped dolphin	KW2010008	Robust	48.2	1.20	0.010	<0.010	<0.010	0.010	14.5
Spinner dolphin	KW2007004	Thin	61.1	0.800	0.260	0.030	1.00	1.29	6.82
Spinner dolphin	KW2007005	Thin	46.0	0.500	0.900	1.62	2.50	5.02	6.24
Spinner dolphin	KW2008002	Robust	53.4	1.20[a]	0.800	4.57	<0.010	5.37	12.7
Spinner dolphin	KW2008004	Robust	32.7	1.30	0.230	<0.010	<0.010	0.230	21.1
Spinner dolphin	KW2009004	Robust	69.4	1.30	2.15	<0.010	0.080	2.23	9.41
Spinner dolphin	KW2010006	Robust	76.8	1.50	0.040	<0.010	<0.010	0.040	14.2

[a]Blubber thickness recorded did not indicate the location of measurement.

CYP1A1 = Cytochrome P450 1A1; EC = endothelial cell count in blubber tissue; TEO = total extractable organic content; NA = data not available.

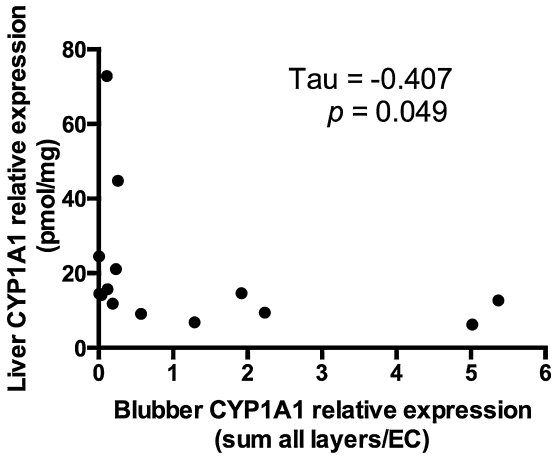

Figure 3. Relationship of cytochrome P450 1A1 (CYP1A1) relative expression between liver and blubber from stranded Hawaiian Island *Stenella* ($n = 14$, 3 species). Liver CYP1A1 expression is shown in pmol/mg, and blubber expression is shown as the sum of all layers expression/endothelial cell count (EC). The correlation coefficient (Kendall's Tau) and p value were generated using the NADA package in R [29].

Paired liver and blubber samples were available for 23 individuals. To limit the variability caused by size and physiology in this diverse cetacean suborder, we limited our examination to 14 individuals from the genus *Stenella*. When the liver-blubber CYP1A1 relationship was explored across all species ($n = 23$), no significant correlation was observed. However, when the liver and blubber CYP1A1 levels were compared among individuals within the *Stenella* genus ($n = 14$), CYP1A1 expression in liver was negatively correlated ($p = 0.049$, Kendall's Tau $= -0.407$) with CYP1A1 in blubber (Figure 3). Perhaps more importantly, a negative correlation was apparent between lipid percentage in the blubber and CYP1A1 relative expression in the liver ($p = 0.020$, Kendall's Tau $= -0.352$, $n = 23$), showing that as lipids decrease in cetacean blubber, the expression of CYP1A1 increases in the liver.

It is tempting to attribute this negative correlation between lipid content and liver CYP1A1, and perhaps *Stenella* blubber CYP1A1 with liver CYP1A1, as evidence of contaminants mobilizing between tissues, particularly in the context of stressed cetaceans on the verge of stranding. Stranded cetaceans often present with empty stomachs, so it is possible that these animals did not feed regularly for some time period prior to death and may have drawn on lipid energy reserves in blubber. If this scenario occurred, then lipophilic contaminants were likely mobilized from blubber storage, passed through the endothelial cells of the blubber, and were transported to the liver where phase I metabolism primarily occurs. Although little is known about toxicokinetics of POPs in cetaceans, blood may facilitate transport of lipophilic contaminants throughout the body, and the distribution of POPs results in tissue-specific contaminant exposure due to lipid dynamics [34]. Blubber endothelial cells are unlikely to have the metabolic capacity to respond to contaminants to the extent possible in the liver, and might even experience localized suppression as observed in animals exposed to extremely high levels of contaminants [8,33]. Therefore, contaminant classes would be more likely to show a significant positive relationship with CYP1A1 expression in the liver, because these stranded animals would be actively attempting to metabolize and remove contaminants from the body (Figure 2 and Figure 3). In fact, it has been shown that cetacean CYP1A1 activity in liver microsomes is higher than that in endothelial cells of the blubber [35]. Lastly, it should be noted that although some studies have shown statistically significant relationships between contaminants measured in the blubber and CYP1A1 expression measured in the blubber of cetaceans [8,28], stronger relationships have been found between contaminants in the blubber and CYP1A1 expression in the liver of beluga whales [21] and largha seals (*Phoca largha*) [36]. This contaminant mobilization hypothesis requires further investigation and is the first time evidence of this physiological effect is presented in stranded cetaceans for this region.

It is also important to acknowledge the inter- and intraspecific differences in responses that undoubtedly exist, but cannot be easily addressed with our data set that contains many species but few representatives within species. The 3 species of the *Stenella* genus are the exception. Interestingly, Bachman et al. [1] report that among stranded cetaceans from the Pacific Islands region, striped dolphins have among the highest blubber concentrations of $\sum$PCBs (median 11 600 ng/g lipid, $n = 6$), followed by spotted dolphins (median 2380 ng/g lipid, $n = 3$), and spinner dolphins (median 2090 ng/g lipid, $n = 10$), which are considered medium to low in their general POP concentration ranges. Striped dolphins, with generally high levels of POPs, have high levels of CYP1A1 liver expression with lower levels of CYP1A1 blubber expression, whereas the spinner and spotted dolphins, with generally lower POP levels, have higher levels of CYP1A1 blubber expression with lower levels of CYP1A1 liver expression (Table 3).

CONCLUSIONS

The present study examined a physiological effect resulting from previously determined POP exposure to stranded cetaceans from the Pacific Islands region. Liver CYP1A1 relative expression was analyzed from 39 cetaceans (16 species) over a 15-yr period. These results from an incredibly diverse suborder of marine mammals continue to suggest that there are common responses to contaminant exposure among mammals. The CYP1A1 expression in the liver correlated positively with the majority of POP compound classes examined, supporting the use of CYP1A1 as a biomarker in stranded Pacific cetaceans. The CYP1A1 was also examined in blubber tissues, but only one significant trend was found between blubber CYP1A1 expression (deep layer) and POP concentrations ($\sum$HCHs) and therefore does not support the use of this biomarker for POP exposure in blubber tissues of stranded cetaceans that may have experienced contaminant mobilization. Specific to our investigation of the genus *Stenella*, a negative trend was apparent between liver and blubber CYP1A1 expression for these stranded cetaceans, which invites further questions about the impacts of poor nutrition, starvation, or even disease progression on the use of CYP1A1 as a biomarker. Investigation into dioxin, PAH and planar PCB exposure for these same stranded animals would also assist in further understanding the quality of CYP1A1 as a biomarker of exposure and potential effect in cetaceans from the tropical Pacific basin. The present study should provide a baseline for future toxicological work in this understudied region of the Pacific.

SUPPLEMENTAL DATA

Table S1. (19 KB DOCX).

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Disclaimer—Certain commercial equipment, instruments, or materials are identified in the present study to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Data availability—Data, associated metadata, and calculation tools are available on request from the authors (bjensen@hpu.edu).

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