

*Hazard/Risk Assessment*PROBABILISTIC RISK ASSESSMENT OF REPRODUCTIVE EFFECTS OF
POLYCHLORINATED BIPHENYLS ON BOTTLENOSE DOLPHINS
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Abstract—High levels of polychlorinated biphenyls (PCBs) have been reported in the tissues of some species of marine mammals. The high concentrations are of concern because a growing body of experimental evidence links PCBs to deleterious effects on reproduction, endocrine homeostasis, and immune system function. Much of the recent research has focused on determining the exposure of marine mammal populations to PCBs, but very little effort has been devoted to the actual risk assessments that are needed to determine the expected impacts of the documented exposures. We describe a novel risk assessment approach that integrates measured tissue concentrations of PCBs with a surrogate dose–response relationship and leads to predictions of health risks for marine mammals as well as to the uncertainties associated with these predictions. Specifically, we use PCB tissue residue data from three populations of bottlenose dolphins (*Tursiops truncatus*), study the feasibility of published dose–response data from a surrogate species, and combine this information to estimate the risk of detrimental reproductive effects in female dolphins. Our risk analyses for dolphin populations near Beaufort (NC, USA), Sarasota (FL, USA), and Matagorda Bay (TX, USA) indicate a high likelihood that reproductive success, primarily in primiparous females, is being severely impaired by chronic exposure to PCBs. Excess risk of reproductive failure, measured in terms of stillbirth or neonatal mortality, for primiparous females was estimated as 60% (Beaufort), 79% (Sarasota), and 78% (Matagorda Bay). Females of higher parity, which have previously off-loaded a majority of their PCB burden, exhibit a much lower risk.

Keywords—Risk assessment Polychlorinated biphenyls Marine mammal Bottlenose dolphin Reproduction

INTRODUCTION

Alarming high levels of polychlorinated biphenyls (PCBs) and other persistent organochlorine contaminants (POCs) have been reported from tissues of marine mammals over the past several decades [1–8]. The high tissue concentrations are a consequence of these animals' high trophic level and lipid-rich blubber that acts as a reservoir for lipophilic chemicals, leading to retention and accumulation of contaminants over time. The incorporation of PCBs is augmented by the inability of marine mammals to eliminate lipophilic compounds through water–blood exchange (via gills), which is the dominant mechanism for elimination in other aquatic organisms such as fish [9,10]. The high PCB concentrations have prompted concern for the well-being of many marine mammal populations, particularly in light of the growing body of experimental evidence linking PCB exposure to deleterious effects on reproduction [11–13], endocrine homeostasis [14] (for review, see [15]), and immune function [16,17]. Adverse effects of PCBs have been clearly demonstrated experimentally in terrestrial mammals [11,12,18–20] and have been suggested to occur in marine mammals as well (for review, see [21]). Specifically, PCBs and associated DDT-like compounds have been linked with premature pupping in sea lions [22], and decreased fecundity, implantation failure, and sterility have

been associated with PCBs in harbor and ringed seals [14,23,24]. Reduced reproductive capacity due to POC exposure has also been proposed as the primary cause for the lack of recovery of the St. Lawrence beluga whale population [2]. Heightening the concerns for marine mammal health are recent reports of disease outbreaks in various populations of dolphins and other marine mammals that have been directly or indirectly linked to high contaminant exposure [4,5]. Recovery of the affected populations could be threatened by the risk of reduced reproductive rates associated with the same pollutant exposure.

Knowledge of the potential health risks associated with exposure to POCs is essential for the effective formulation of conservation and management plans. Recent research has focused on the measurement and analysis of environmental exposures in marine mammal populations. However, systematic, quantitative risk assessment efforts, which are needed to interpret the significance of the reported exposures, have been lacking. A major complication in predicting or estimating risks for marine mammals is the high degree of uncertainty resulting from the lack of dose–response information and the large natural variability in exposures among individuals. Uncertainties in assessing effects are unavoidable because of political and legal limitations imposed on experimental studies for protected species and the complex logistics involved in population studies for these generally long-lived and wide-ranging aquatic

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mammals. As a result, formal risk assessments are complicated and therefore scarce.

Our research addresses this deficiency with a probabilistic approach to risk characterization that yields quantitative estimates of the risks themselves and also of their associated uncertainties. We focus on the risk of fetal and neonatal mortality associated with maternal exposure to PCBs because evidence for this type of adverse reproductive effect has been presented in numerous studies of both terrestrial and aquatic mammals [11–13,19,25–27]. Specifically, we combine PCB tissue residue data collected from three populations of bottlenose dolphins with a dose–response relationship derived from experimental studies on a surrogate mammalian species. This integration of data from different sources with a mathematical model allows us to assess reproductive endpoints and, to some degree, immunological effects. To determine overall uncertainty in predicted risks, the uncertainties resulting from the assessments of exposure and dose response are propagated through the risk characterization process using Monte Carlo analysis.

APPROACH AND METHODS

Study populations

Three dolphin populations were targeted for risk assessment based on recent epizootic events or suspected high pollutant exposure. The PCB exposure data was obtained as part of the ongoing dolphin health assessment studies being conducted by both the National Marine Fisheries Service and the Chicago Zoological Society (USA). In 1982, scientists with the Chicago Zoological Society's Sarasota Dolphin Research Program began collecting blood samples and body condition measurements from free-ranging bottlenose dolphins in Sarasota Bay in order to evaluate the health of a resident population that has been under study since 1970 [28,29]. In 1988, studies were initiated to investigate the relationships of environmental contaminant concentrations in blood and milk of these animals to health, body condition, and reproduction [30]. Matched blubber samples have been collected since 1997. Building on the model established in Sarasota Bay, in 1992 the National Marine Fisheries Service began a program to collect blubber biopsy samples from live dolphins of key populations and to relate contaminant concentrations to health. The biopsy samples are analyzed for a suite of organochlorine contaminants, including 15 PCB congeners and a variety of persistent pesticides, such as chlordane and DDT-like compounds.

Beaufort

From early June of 1987 until March of 1988, over 700 bottlenose dolphins washed ashore along the U.S. Atlantic coast from New Jersey to Florida (USA). The exact number of mortalities is unknown since most animals were never recovered, but it was estimated that over 50% of the putative coastal migratory stock died during this period [31]. The cause of this massive epizootic event is still somewhat controversial. Both infectious disease (morbillivirus) and brevetoxin poisoning have been proposed as possible factors [32,33]. High organochlorine levels were found in the stranded animals and could have been a contributing factor for the event but are not believed to have been the initiating factor (K. R. Wang et al., National Oceanic and Atmospheric Administration Technical Memorandum, National Marine Fisheries Service-OPR-4). Because of these excessive mortalities, the Atlantic coastal mi-

gratory dolphin stock was listed as depleted under the Marine Mammal Protection Act in 1993 [34]. The depleted status and the potential role of POCs in the 1987–88 epizootic make the Atlantic coastal stock a high research priority for risk assessment efforts.

The exact boundaries and migratory patterns of the Atlantic coastal stock are somewhat uncertain, and multiple stocks appear to exist (A.A. Hohn, National Marine Fisheries, Beaufort, NC, USA, unpublished data; P.E. Rosel, National Marine Fisheries Service, Charleston, SC, USA, unpublished data). During the summer, the primary concentration of migratory dolphins occurs from North Carolina northward to New Jersey (Waring et al., National Oceanic and Atmospheric Administration Technical Memorandum, National Marine Fisheries Service-NE-153). In July 1995, the National Marine Fisheries Service conducted a live capture of dolphins near Beaufort to assess health and to obtain blubber biopsies for analysis of POC concentrations. The results of this effort are included in our risk assessment.

Matagorda Bay

An unusual increase in bottlenose dolphin mortality was observed in the Gulf of Mexico between January and May 1990 [35]. In this event, approximately 350 dolphins stranded on beaches from Texas to Florida, with a portion of these strandings occurring in Matagorda Bay. Another unusual increase in the number of strandings occurred in 1992 but was confined to a smaller geographic area that also included Matagorda Bay. Again, the exact cause of the increased mortality was not determined for either of the two stranding episodes, but high concentrations of PCBs and other POCs were found in tissues of the animals that stranded in 1990 [36]. Because of the magnitude of these two events and the uncertainty of the cause, in 1992 the National Marine Fisheries Service conducted live captures of dolphins in Matagorda Bay to assess the health of the affected population and to obtain blubber biopsies for analysis of contaminant concentrations.

Sarasota

The final population considered for risk assessment resides near Sarasota. In contrast to the Matagorda Bay and Beaufort dolphins, this population has not experienced anomalous disease or mortality episodes. However, it is of particular interest for two reasons. First, the Sarasota population has been the focus of an ongoing observational study for more than 30 years [28] and is likely the best-studied small cetacean population in the world. As such, this population provides a unique opportunity to test predicted risk estimates against observations and reproductive history of known individuals over time. Second, the habitat of the Sarasota population is in close proximity to a densely populated coastline within a bay that has known pollution problems [37,38].

Blubber biopsies were obtained from animals in the Sarasota Bay area as part of the annual health assessment conducted by the Chicago Zoological Society in 1997, 1998, and 1999. Contaminant residues were analyzed using the same laboratory that conducted the analyses of the Matagorda Bay and Beaufort samples. This is important to note because it eliminates the problems associated with possible interlaboratory variability.

Exposure assessment

Field methods for the live capture of dolphins have been previously described [39]. Wedge biopsies measuring approx-

imately 3.5 cm long by 1.5 cm wide by 1.0 cm deep and weighing about 1 g were taken from the left side of each animal at a site about 10 cm behind and 10 cm below the posterior aspect of the dorsal fin. The site was prepared with an antiseptic scrub, and a local anesthetic was injected in an L-block configuration, approximately 4 cm ventral and anterior to the intended biopsy site. A sterile scalpel and forceps were used to cut and extract the sample.

The samples were analyzed by the National Ocean Service's Center for Coastal Environmental Health and Biomolecular Research at Charleston (SC, USA). Blubber samples were macerated with sodium sulfate and, after the addition of the internal standards, extracted by accelerated solvent extraction. Lipids were removed from the pesticides and other organic contaminants by gel permeation chromatography. Interfering polar compounds were removed by Florisil (U.S. Silica, Berkeley Springs, WV, USA) chromatography. The samples were then analyzed by capillary-gas chromatography electron impact mass spectrometry. Standard reference material ([SRM] 1945 whale blubber) was analyzed for each group of samples and compared with reference values for quality assurance. The laboratory participated in the Intercomparison Exercise for Persistent Organochlorine Contaminants in Marine Mammal Blubber sponsored by the National Institute of Standards and Technology to ensure quality control and interlaboratory comparison capability. Although the concentrations of 15 individual congeners were determined, the calculation of total PCBs was made by comparison with an Aroclor 1254 (Monsanto Chemical, St. Louis, MO, USA) envelope. This method of total PCB calculation has been commonly employed in the past for toxicological studies (for discussion, see [40]). For consistency and comparability of results, we employed this method as well.

The ages for some Sarasota animals were known from observed birth dates. For the remainder of the Sarasota animals and for all the animals from other sites, age was estimated from counts of the number of growth layers in teeth [41].

Age and gender related trends in POC residues have been demonstrated in dolphins and other cetacean species [3,8,42,43]. The POC burdens in males reportedly remain stable or increase with age. Conversely, contaminant concentrations in sexually mature females generally decline, a trend that is attributed to the transfer of contaminants to offspring during gestation and, more notably, during lactation [30]. For instance, Cockcroft et al. [3] found a sharp decrease in contaminant burden following birth and lactation in primiparous bottlenose dolphins. The decrease in POC residues following subsequent births was less discernible, indicating that females impart the majority of their contaminant load to their firstborn calf. In addition to the decline in contaminant concentrations in reproductively active females, Tanabe et al. [42] reported increasing concentrations in sexually immature female and reproductively senescent female short-finned pilot whales (*Globicephala macrorhynchus*). However, this relationship in reproductively inactive females was not supported by a later study of long-finned pilot whales (*Globicephala melaena*) [43].

A crucial component of any health risk assessment is obviously the extent of exposure. Given the anticipated differences in contaminant concentrations between sexually immature and mature females yet an indistinct relationship between age and PCB residue within the two groups, we chose to model exposure within the female population with two sep-

arate probability distributions, one for each group. Sexual maturity is believed to occur in females of this species at about 8 to 10 years of age [44]. Since individual reproductive histories were not known for the Matagorda Bay or Beaufort study animals, we defined broad age classes for juvenile females 10 years or less (assumed to be nulliparous) and adult females greater than 10 years.

As typical, the distributions of contaminant concentrations were assumed to be normal following a log transformation. Thus, lognormal distributions were fit for total PCB residue values, stratified by populations and the two age classes.

Hazard assessment

Kannan et al. [45] recently derived a threshold concentration for adverse health effects from PCB exposure in the blubber of marine mammals. This threshold, at a level of 17 $\mu\text{g/g}$ PCBs lipid weight, was based on experimental studies of both immunological and reproductive effects in seal, otter, and mink. As a preliminary hazard assessment, we compared this threshold value to the total PCB blubber concentrations from the sampled dolphin populations, which we had first fitted with lognormal distributions.

Effects assessment

Quantitative relationships between PCB tissue residues and adverse reproductive effects have not yet been established in dolphins. This is due primarily to the ethical and logistical constraints inherent in experimental studies of this protected species. The lack of data for dolphins forces us to rely on surrogate species for estimating likely levels of effect. The mink (*Mustela vison*) has been suggested as a potential surrogate [45], primarily because of similarities in reproductive physiology with some marine mammals. Reproductive failure in mink related to PCB exposure has been demonstrated in numerous studies [12,26,46–48]. Typically, ovulation and nidation occur, but the fetuses die during gestation or shortly after birth [27,49,50]. Since mink are a relatively sensitive species with regard to PCB-related reproductive effects, they serve as a useful model for conservative estimations of effects. This is particularly desirable when endangered or protected species are the focus of the risk assessment, and for this reason we selected mink as the surrogate species on which to base the calculation of likely reproductive effects.

Results from two mink feeding studies with similar protocols [51,52] and that employed naturally contaminated prey from Saginaw Bay (MI, USA) as the PCB exposure vector were combined to produce a toxicity data set with eight treatment levels spanning a broad range of dose levels. The studies were selected because along with dosing levels and associated percentage of kit mortality, PCB concentrations in the minks' livers were reported. This allowed us to model the toxic effects as a function of tissue concentration rather than oral dose or dietary concentration, thus allowing for direct comparison with the measured PCB tissue concentrations from the dolphins. The direct comparison of tissue residues is advantageous in that it circumvents some of the uncertainty due to interspecies differences in absorption and metabolism.

Excess risk of offspring mortality was calculated on the basis of the general equation defined by Hoel [53] to adjust for control group response frequency:

$$P_e = \frac{P_t - P_c}{1 - aP_c} \quad (1)$$

where = decline in fecundity due to PCBs = proportion of offspring not surviving in the control group = proportion of offspring not surviving in the exposed group = proportion of control response caused by an independent mechanism

The control response was assumed to be completely independent from that of the test dose response ($a = 1$), yielding the most conservative result. This form of Equation 1 with $a = 1$ is also known as Abbot's correction [54].

Tissue residues and adjusted response frequencies were used to estimate a concentration–response relationship for reproductive failure (specifically, stillbirth, and neonatal mortality) versus lipid-normalized PCB liver residue using the generalized linear model (GLM) framework with a probit link function and a binomial error distribution. Kerr and Meador [55] have described use of the GLM framework for modeling dose–response data. The advantages of this type of model over the more generally applied probit model are twofold. First, a GLM allows extreme responses (0 and 100%) to be included in the model without approximation for infinitely negative or positive values. Second, with a GLM, a measure of associated uncertainty in parameter estimates can easily be obtained in the form of standard errors. Applying the GLM, the inverse probit link specifies the predicted response for a given PCB concentration:

$$\hat{p}(x) = \Phi(\beta_0 + \beta_1 \cdot x) \quad (2)$$

where x is the given PCB concentration, $\Phi(y)$ is the cumulative standard normal distribution, and β_0 , β_1 are the estimated parameters of the GLM.

Estimation of tissue concentration

Restum et al. [52] report total PCB concentration from pooled liver samples for each dose group. In the study by Heaton et al. [51], PCB liver concentrations were reported for the control and lowest-dose group (as no-observed-adverse-effect level [NOAEL] and lowest-observed-adverse-effect level [LOAEL] values), but for the two higher-dose groups, only dietary concentrations were reported. Therefore, a mechanism for predicting tissue residue based on the reported dietary concentration was needed in order to estimate liver concentrations for the two higher-dose groups. To accomplish this, we used the model form described by Leonards et al. [56]. This model, in its original form, was used to determine body concentrations of individual PCB isomers based on dietary concentrations of the isomers in feed, estimated feed consumption, and isomer-specific assimilation and elimination parameters. We employed this same equation to predict total PCB liver concentration based on dietary concentration of total PCBs rather than the concentration of individual isomers and total PCB absorption and elimination parameters rather than separately specified parameters for each individual isomer. The implementation of the model then required three parameters: assimilation efficiency of total PCBs, estimated daily feed consumption (g/g/d), and an elimination rate constant for total PCBs. We estimated assimilation efficiency of total PCBs based on the absorption rate of Aroclor 1254 in ferrets (*Mustela putorius furo*) (85.4%) reported by Bleavins et al. [57]. The total PCB elimination rate constant was estimated using a nonlinear optimization algorithm based on paired dietary doses and associated liver concentrations [51,52]. Mathcad 2001 (MathSoft, Cambridge, MA, USA) was used to perform the optimization of this parameter value and produced an estimate of 0.087 for the elimination rate of total PCBs. This is near the midrange

of elimination rates reviewed by Leonards et al. [56] for individual congeners (range 0.007–0.231).

Since the model estimates a wet-weight tissue concentration, a conversion to lipid weight was still needed. A factor for converting wet-weight tissue concentration to a lipid-weight concentration was derived from ratios of values reported in [51]. The calculated factor was 0.047, implying an average lipid weight of liver of 4.7% for mink. This is in agreement with the average lipid content of 5% reported by Poole et al. [58] for populations of wild mink from the Northwest Territories, Canada.

Risk characterization

Risk characterization is the phase of risk assessment where the results of the exposure and quantitative effects assessments are integrated to provide an estimate of risk for the population under study. In this case, it entails combining the exposures, measured as blubber concentrations from the various dolphin populations, with the quantitative concentration–response relationship between tissue residue and associated offspring mortality determined from the experimental studies of mink.

Risk at a specific PCB concentration, X , can be calculated as the proportion of the group expected to have that tissue concentration multiplied by the conditional probability of offspring mortality, given concentration X . This can be expressed symbolically as

$$R_x = P(X)P(E|X) \quad (3)$$

where R_x is the risk at a specific concentration X , $P(X)$ is the probability of having tissue concentration X , and $P(E|X)$ is the conditional probability of the adverse effect, given tissue concentration X .

The overall expected risk for a group may be computed as the sum of the risks for all possible X s. Specifically, since the tissue concentrations within a group or subpopulation are distributed normally after log transformation and the responses follow Equation 2, the overall group risk can be estimated as

$$R = \int_{-\infty}^{\infty} \frac{1}{\sigma_e \sqrt{2\pi}} \exp\left[-\frac{1}{2}\left(\frac{\log X - \mu_e}{\sigma_e}\right)^2\right] P(E|X) d \log X$$

or, substituting in Equation 2,

$$R = \int_{-\infty}^{\infty} \frac{1}{\sigma_e \sqrt{2\pi}} \exp\left[-\frac{1}{2}\left(\frac{\log X - \mu_e}{\sigma_e}\right)^2\right] \times \left(\int_{-\infty}^{\beta_0 + \beta_1 X} \frac{1}{\sqrt{2\pi}} \exp\left[-\frac{1}{2}(D)^2\right] d \log D \right) d \log X \quad (4)$$

where μ_e and σ_e are mean and standard deviation of the log-transformed exposures, respectively; β_0 and β_1 are the fitted parameters for the concentration–response function; and R is the estimated fraction of the group that is expected to suffer reproductive failure.

Analysis of uncertainty

Uncertainty arises from estimation of both exposure and effects. In order to quantify this uncertainty and its impact on the estimation of expected risk, we implemented a Monte Carlo simulation that includes input distributions for the parameters of the derived concentration–response function as well as for estimated exposure parameters. The implemented parameter distributions are summarized in Table 1 and described in the

subsequent sections. Fifty thousand executions of the Monte Carlo simulation were performed. A confidence interval for expected risk was determined on the basis of the 2.5th and 97.5th quantiles of the simulation results. A risk curve was generated from the cumulative distribution of simulation outcomes. The x axis of the risk curve can be interpreted as a magnitude of effect (a percentage of the given population expected to suffer adverse effect), and the y axis can be interpreted as the probability that an effect of at least that magnitude will occur. The simulation was implemented using Mathcad 2001.

Exposure

For the exposure assessment, the number of samples from each population was small, primarily because of the extensive effort involved in obtaining dolphin tissue samples. This was particularly true after stratification into age and gender classes. Limited sample sizes are a general problem with protected species and are unlikely to be circumvented with increased sampling efforts. While representing the exposure of a given class as a distribution rather than a single point estimate captures the variability among individuals, the limited sample sizes produce uncertainty in the estimation of the distribution parameters, and this uncertainty should also be accounted for in estimation of risk. We employed a bootstrap approach to estimate the distribution of parameters (μ_e , σ_e) for the calculated exposure distributions. For each iteration of the Monte Carlo simulation, a bootstrap sample was drawn from the appropriate exposure data set, and estimates of the exposure distribution parameters were fit. By this method, the estimates for the two parameters, μ_e and σ_e , were treated as paired values, accounting for potential correlation.

Additionally, since for the risk characterization we are combining the dolphins' exposures, measured as blubber concentrations, with a concentration–response relationship derived for liver residue, a question arises as to the comparability of the two tissue concentrations (between blubber and liver). Contaminant concentrations in blubber, described as wet weights, have been shown to be high in comparison to other tissues, primarily because of the blubber's high lipid content. However, when values are based on total lipid weight, the concentrations are more comparable between tissues [59–61], and therefore lipid-normalized PCB concentrations in blubber can be used as an indicator of concentrations in other tissues, such as liver. We have assumed a tissue conversion factor (blubber/liver ratio) equal to 1.0. However, studies of cetaceans have reported values both less than and greater than 1.0. A study of beluga whales from the St. Lawrence Estuary [2] reported an average PCB blubber/liver conversion factor of 1.19, but values for the 15 sampled whales ranged from 0.04 to 3.24, indicating some degree of variability between individuals. To capture this interindividual variability, we plotted and analyzed the blubber/liver conversion factors reported in [2] to identify a probability distribution that reasonably represents the data. A gamma distribution was determined to provide the best fit, with a scale parameter of 0.85 and a shape parameter of 1.40 obtained using numerical approximation to maximum likelihood estimates. The gamma distribution, representing the variability of blubber/liver conversion factors, was incorporated into the Monte Carlo analysis.

In addition, we incorporated distributions to represent the uncertainty with regard to the true values for the gamma distribution parameters. The distributions for the gamma param-

eters were determined on the basis of multiple blubber/liver conversion factors that have been reported for cetaceans [2,62,63]. Shape and scale parameters were calculated using the method of moments from the blubber/liver ratio mean and standard deviation reported from each study. Since parameter estimates were obtained from only three studies, determination of an appropriate distribution was difficult. However, lognormal distributions have been found to reasonably represent gamma scale and shape parameters in other simulation analyses [64]. Lognormal distributions were therefore selected, and a bivariate distribution was fit to the collection of paired gamma parameters in order to capture the correlation that may exist between the shape and scale values.

Concentration–response uncertainty

In applying concentration–response relationships derived from experimental studies, we must consider the limitations of the data and account for the inherent uncertainty that arises from a number of sources, including the limited number of treatment groups and limited sample size within treatment groups. To account for this uncertainty, we constructed distributions for the regression coefficients (β_0 , β_1) of the GLM concentration–response functions. Regression coefficients obtained in GLM models are asymptotically normally distributed. We determined a bivariate normal distribution for the coefficients on the basis of the estimates of (β_0 , β_1), their standard errors, and the correlation coefficient between them, and incorporated this distribution into the Monte Carlo simulation.

One key source of uncertainty in the concentration–response analysis could not be included in the simulation. Uncertainty and/or variability were not considered for the reported liver concentrations. This was unfortunate but unavoidable since liver concentrations from the published studies were reported only as average values or were based on pooled samples. As a result, the risk curves and confidence limits reported here do not incorporate this source of uncertainty.

RESULTS

Exposure assessment

Polychlorinated biphenyl residues measured from all sampled populations confirmed the expected trends with respect to both age and gender (Fig. 1). Adult males and juveniles exhibited the highest concentrations, while mature females, which are presumably reproductively active, maintained much lower PCB levels. Measurements for mature males were limited, so age-related trends in males suggested by previous studies [8] could not be confirmed. For females, a definite drop in contaminant concentration can be seen after about 10 years of age, confirming that the proposed separate distributions for immature and mature females were appropriate. Exceptions to this general observation occur in the Matagorda Bay samples: FB515 and FB501 are 12 and 17 years of age, respectively, and so were originally classified as mature females. However, their PCB concentrations are more suggestive of nulliparous females that have not yet off-loaded contaminants to a calf. Given this observation, these two females were reclassified as members of the immature group. While the reclassification of these two samples does not significantly alter the estimated exposure parameters for the immature group, it does decrease the estimated exposure level for the mature class, thus leading to less conservative risk estimates for this class.

Two of the older females, FB519 and FB523, from Mata-

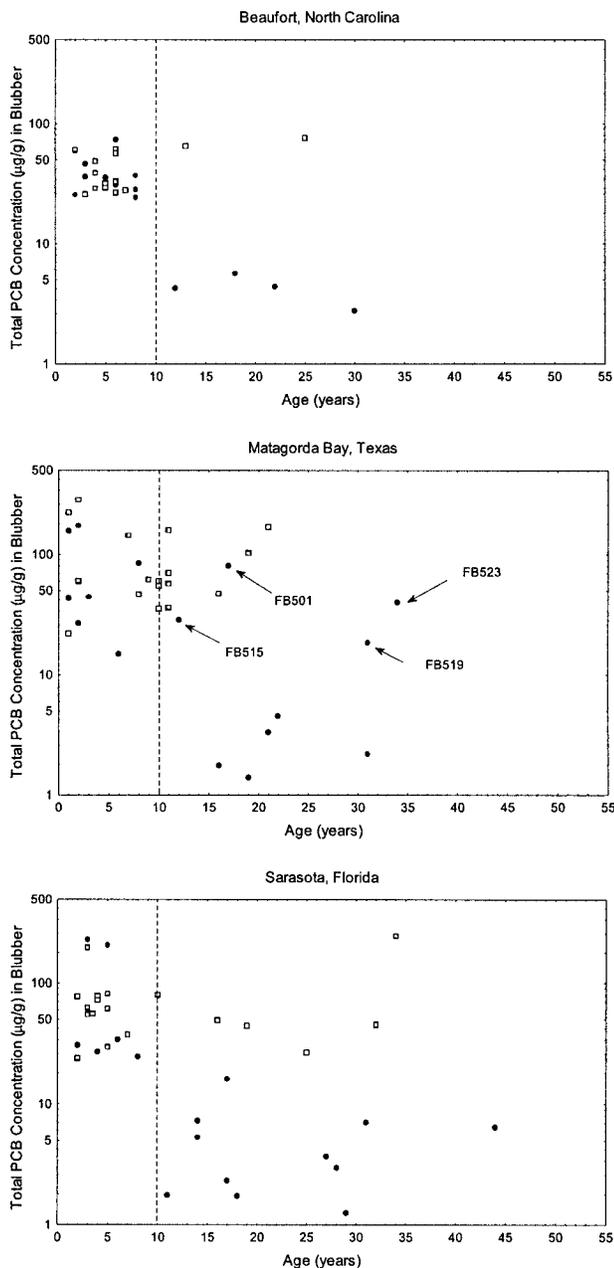


Fig. 1. Age versus total polychlorinated biphenyl (PCB) blubber concentrations. Filled circles represent females, open squares represent males. Vertical dashed line represents threshold for separation between immature and mature age classes for females. Mature males and immatures exhibit the highest concentrations, while mature females maintained much lower PCB levels. The exceptions are FB515 and FB501, 12 and 17 years of age, respectively, whose PCB concentrations are more similar to immatures than other mature females. Two of the older females from Matagorda Bay (TX, USA), FB519 and FB523, also exhibit higher-than-expected concentrations as compared to others in their age class.

gorda Bay also exhibit higher-than-expected PCB concentrations as compared to others in their age class. Neither of these animals was pregnant or lactating at the time of sampling, and it is likely that these two animals were not reproductively active. However, the underlying cause for the inactivity can only be speculated. Given the age of the animals and no additional information, the classifications for FB519 and FB523 were left as mature females.

Given the observed patterns in contaminant concentrations

and because no gender-related differences in tissue concentrations were observed for dolphins ten years of age or less ($p = 0.49$), it seemed valid to combine samples from both males and females in this group for the estimation of the contaminant distribution among immatures. Summary statistics of the measured PCB concentrations are shown in Table 2 for the three study populations.

The derived exposure distributions for juveniles and adult females within each population are shown in Figure 2. The distributions of concentrations in juveniles almost entirely exceed (>94% for all populations) the threshold value established by Kannan et al. [44], while the distributions for mature females fall primarily below the threshold, suggesting a much greater health risk for immature animals. The distribution of PCB concentrations for the adult females from Matagorda Bay has a much greater spread than the Sarasota and Beaufort distributions. This is due primarily to the two previously mentioned older females that exhibited uncharacteristically high blubber concentrations. In reality, the distribution of concentrations for mature females may be bimodal with a lesser mode for reproductively active females and a higher mode for reproductively senescent or otherwise reproductively inactive females.

Distributions for adult males, while based on a very limited number of samples, indicate risks comparable to those for juveniles, with a 96 and 95% probability of exceeding the proposed threshold value for Sarasota Bay and Matagorda Bay populations, respectively. No estimate was made for the Beaufort population since only two samples were obtained from adult males.

Estimation of concentration–response function

The GLM model with a probit link model and binomial error distribution provided an adequate fit for the data (χ^2 goodness of fit, $p > 0.10$). Based on this concentration–response function (Fig. 3), the calculated median effective concentration (EC50) is 33 $\mu\text{g/g}$ total PCBs lipid weight (liver or blubber), or 1.55 $\mu\text{g/g}$ total PCBs in liver on a wet-weight basis.

The threshold concentration for PCBs in marine mammal blubber established by Kannan et al. [44] was calculated on the basis of reported NOAEL and LOAEL values. A threshold value can also be estimated on the basis of the fitted dose–response model. This approach is comparable to the use of a benchmark dose [65], an alternative method for reference value calculations that relies on a dose–response model to calculate a statistical lower confidence limit on a dose producing some predetermined increase in response rate (increased risk). The selected level of risk is typically not far below the range of observed data (e.g., 1, 5, or 10% risk level) [66] and has been suggested to be at least 10% for proportional data, such as prenatal deaths [67]. The EC10 value calculated from the fitted dose–response model is 14.8 $\mu\text{g/g}$ total PCBs lipid weight, with a 95% confidence interval of 11.0 to 18.0. Following the benchmark dose approach, the threshold concentration, estimated as the lower confidence interval of the EC10, would be 11.0 $\mu\text{g/g}$. A less conservative threshold concentration based on the point estimate of the EC10 would be 14.8 $\mu\text{g/g}$, which is very close to the threshold value of 17 $\mu\text{g/g}$ proposed by Kannan et al. [45].

Risk estimates for dolphin populations

The point estimate of risk for reproductive failure for each age class within each sampled population is given in Table 3.

Table 1. Input variables and distributions used in the Monte Carlo simulations for analysis of uncertainty. (I) indicates immature class, (M) indicates mature class

Description	Variability/ uncertainty	Distribution	Parameters ^a
PCB ^b tissue concentration	V	Lognormal	μ_e , σ_e (calculated from a bootstrap sample for each iteration)
Sampling distribution for μ_e , σ_e of PCB tissue concentration	U	Nonparametric bootstrap	Quartiles Beaufort (NC, USA) $\mu_e = (35.27, 37.05, 39.00)$ (I) $\sigma_e = (6.93, 8.98, 7.96)$ (I) $\mu_e = (3.87, 4.17, 4.61)$ (M) $\sigma_e = (0.47, 0.84, 0.62)$ (MC) Matagorda Bay (TX, USA) $\mu_e = (63.35, 72.15, 82.06)$ (I) $\sigma_e = (32.63, 40.01, 48.34)$ (I) $\mu_e = (4.50, 6.67, 9.55)$ (M) $\sigma_e = (3.88, 6.57, 9.99)$ (M) Sarasota (FL, USA) $\mu_e = (58.61, 65.93, 74.11)$ (I) $\sigma_e = (25.46, 37.89, 31.47)$ (I) $\mu_e = (3.69, 5.08, 4.33)$ (M) $\sigma_e = (1.69, 2.81, 2.23)$ (M)
Conversion factor (blubber/liver ratio)	V	Gamma	$\eta \sim \text{lognormal}(\mu_\eta, \sigma_\eta)$ (shape) $\lambda \sim \text{lognormal}(\mu_\lambda, \sigma_\lambda)$ (scale)
Sampling distribution for scale and shape parameters of conversion factor	U	Bivariate lognormal	$\mu_\eta = 0.263$ $\sigma_\eta = 0.480$ $\mu_\lambda = 0.032$ $\sigma_\lambda = 0.370$ $\rho = -0.50$
Sampling distribution for parameters β_0 , β_1 of concentration–response function	U	Bivariate normal	$\mu_{\beta_0} = -2.328$ $\sigma_{\beta_0} = 0.192$ $\mu_{\beta_1} = 0.071$ $\sigma_{\beta_1} = 0.006$ $\rho = -0.86$

^a For nonparametric distributions, quartiles for the distributions are given.

^b PCB = polychlorinated biphenyl.

We define risk as the percentage of the group that is expected to suffer a reproductive failure, such as stillbirth or calf mortality that is in excess of and unrelated to the background incidence. The point estimates were calculated from Equation 4 and ignore uncertainty associated with estimates of exposure and concentration–response parameters. As can be seen, risk for reproductive failure is much higher for the immature females, which represent dolphins that would be reproducing for the first time (primiparous). Risk is particularly pronounced for the primiparous females within the Sarasota and Matagorda Bay populations. In fact, in the Matagorda Bay population, it is expected that for three out of four females, the firstborn offspring will not survive because of maternal exposure to environmental PCBs. It should be stressed that this expected mortality is in excess of the normal background incidence. For example, if we assume a hypothetical baseline rate of successful reproduction of 80%, then in the Matagorda Bay population only 18% of first-time mothers would be expected to produce viable offspring. Risk for subsequent births is substantially lessened, as indicated by the calculated risk for females in the upper age class, once the bulk of a female's PCB burden has already been transferred. In fact, for the Sarasota and Beaufort populations, the estimated risk for the upper age class is only about 2 to 3%. For the females in the upper age class from Matagorda Bay, the risk is somewhat higher (10%), again being influenced by the two older female outliers. Although not shown, it should be noted that if FB501 and FB515 are also included in the mature category, as indicated by their estimated age, the risk for this age class is even higher (20%).

Confidence intervals calculated from the 2.5th and 97.5th quantiles of the Monte Carlo simulation results are also shown in Table 3. These ranges represent the uncertainty in expected risk estimates when both the inherent variability and uncertainty in exposure and concentration–response parameter estimates are considered. The confidence intervals for the expected risk are relatively wide, and sensitivity analyses indicate that this is due primarily to the variability and uncertainty in blubber/liver conversion factors. Unfortunately, this type of uncertainty is difficult to remedy since liver tissue cannot be directly obtained for analysis from live animals. An alternative would be to compare the concentrations in dolphin blubber to adipose tissue concentrations in experimental surrogate species, although the unique structure and function of marine mammal blubber would make this type of comparison questionable. Despite the degree of uncertainty, the high risk for primiparous females is alarming. Even at the lower bound, nearly half (46%) of the Matagorda Bay first-time mothers are expected to fail to reproduce viable calves because of their exposure to PCBs.

Risk curves indicating the estimated probabilities of effects of differing magnitude for both classes of females (assumed primiparous and multiparous) are shown for each of the studied populations (Fig. 4). The plotted probabilities, calculated from the outcome of the Monte Carlo simulation, take into account the uncertainty in estimating risk derived from variability and uncertainty in model parameters. For Matagorda Bay, the probability is 0.97 that at least 50% of calves from primiparous females will not survive. Furthermore, for both Matagorda Bay

Table 2. Summary statistics for total polychlorinated biphenyls (PCBs) in blubber biopsy samples

Population	Age class ^a	n	Total PCB concn. ($\mu\text{g/g}$)		
			Mean	Geometric mean	Standard deviation
Beaufort (NC, USA)	Juvenile	21	38.33	36.28	14.01
	Adult male	2	70.27	70.04	7.92
	Adult female	4	4.24	4.11	1.18
Matagorda Bay (TX, USA)	Juvenile	19	86.24	62.99	74.43
	Adult male	7	91.21	78.45	53.60
	Adult female	7	10.27	4.80	14.50
Sarasota Bay (FL, USA)	Juvenile	19	76.78	59.59	62.94
	Adult male	6	76.18	55.44	84.03
	Adult female	11	5.07	3.84	4.27

^a Juvenile represents both male and female animals 10 years of age or less. Adult male and adult female are greater than 10 years.

and Sarasota Bay, the probability is 0.50 of at least 73% calf mortality for primiparous females.

DISCUSSION

Our analysis indicates a significant risk of fetal or calf mortality for primiparous females in all the studied populations. Risk is particularly pronounced for the Matagorda Bay and Sarasota Bay dolphins. In Matagorda Bay, two of the four dolphins between the ages of 10 to 20 years, assumed to be within reproductive years, maintained contaminant concentrations suggestive of animals that have not yet successfully completed a gestation and lactation cycle. One could speculate that these animals, FB515 and FB501, are experiencing a delay in their first successful reproductive event as a result of their high PCB concentrations. The observation of nonreproductive females several years past the age of expected first birth is certainly consistent with our calculated risk estimates, but the underlying cause of the delay could also be due individual variation, and the attribution to PCB exposure is pure speculation at this point. More detailed information of individual reproductive histories is needed to fully assess the accuracy of the predicted effects.

The assumptions in our analysis are subject to uncertainty that warrants further discussion. First and foremost is the use of a surrogate species for the determination of the concentration–response function. This introduces a high degree of uncertainty because species-specific pharmacokinetic and pharmacodynamic characteristics can strongly influence sensitivity. With regard to pharmacokinetic differences, the consideration of tissue concentrations instead of oral doses circumvents some of the uncertainty associated with interspecies variation in absorption and metabolism. However, the comparison of total PCB concentrations rather than a congener-specific breakdown would not necessarily reveal species-dependent preferential metabolism of specific congeners. In a 1988 study, Tanabe et al. [68] found that the composition of PCB isomers and congeners in tissue varied between species and that the metabolic capacity of marine mammals, particularly small cetaceans, was extremely low compared to those of birds and terrestrial mammals. The results of that study concluded that small cetaceans have no capacity to metabolize a specific group of PCBs that have adjacent nonchlorinated *meta* and *para* carbons in the biphenyl rings. The authors suggest that this metabolic deficiency is attributable to a reduced activity of phenobarbital-type (PB-type) and 3-methylcholanthrene-type (MC-type) enzymes. The activities of PB-type enzymes were ordered from highest to lowest: terrestrial mammals > birds > marine mammals, with small cetaceans exhibiting no activity at all. The

activities of MC-type enzymes were ordered: terrestrial mammals > marine mammals (seals > small cetaceans) > birds. However, the surrogate species that we relied on, mink, was found to be an exception to this general ordering, having much lower activities in both PB- and MC-type enzymes. This similarity in enzyme activity between mink and dolphins makes the comparison of total PCB tissue concentrations more appropriate.

The mechanism(s) of action for PCB-related reproductive toxicity are still not well understood and cannot be assumed to be consistent between species. This being the case, the use of the most sensitive species as a surrogate model is necessary to provide a conservative estimate of probable effects. For PCB-related reproductive toxicity, mink and rhesus monkeys (*Macaca mulatta*) are the most sensitive species for which data are available, as determined by a comparison of LOAELs and NOAELs across studies [69]. An early study of rhesus monkeys [25] reported an incidence of embryonic/fetal loss similar to our estimates of dose–response based on mink data (three of eight animals suffered resorption, abortion, or still-birth associated with mean concentration of 33 $\mu\text{g/g}$ in adipose tissue). Table 4 summarizes PCB concentrations associated with adverse reproductive effects in several mammalian species.

Aside from the desire for a conservative estimate of risk, some evidence suggests that *Tursiops* may very well be among the more sensitive species for PCB toxicity. Although the mechanisms of PCB toxicity have not been thoroughly elucidated, many of the planar PCBs exhibit dioxin-like actions related to their structural configuration and exert some toxic effect via the aryl hydrocarbon receptor (AhR). Species-specific differences in sensitivity to dioxin-like compounds have been shown to be associated to some degree with the binding affinities of the AhR [70,71]. A recent study has suggested that at least one species of cetacean, the beluga whale (*Delphinapterus leucas*), possesses an AhR with binding characteristics similar to that of AhRs from other mammals that are considered sensitive to the toxic effects of this contaminant class [72]. These results support the supposition that cetaceans may be particularly susceptible to the toxicity of at least planar PCBs. Moreover, a comparison of published toxicity data suggests that other marine mammals, such as pinnipeds, exhibit a sensitivity to PCB toxic effects comparable to that of mink [45] and an older study of swine, a species with a closer phylogenetic relationship with Cetacea [73], demonstrated an increase in fetal death associated with adipose PCB concentrations of only 4 to 20 $\mu\text{g/g}$ [19].

Obviously, concentration–response relationships derived

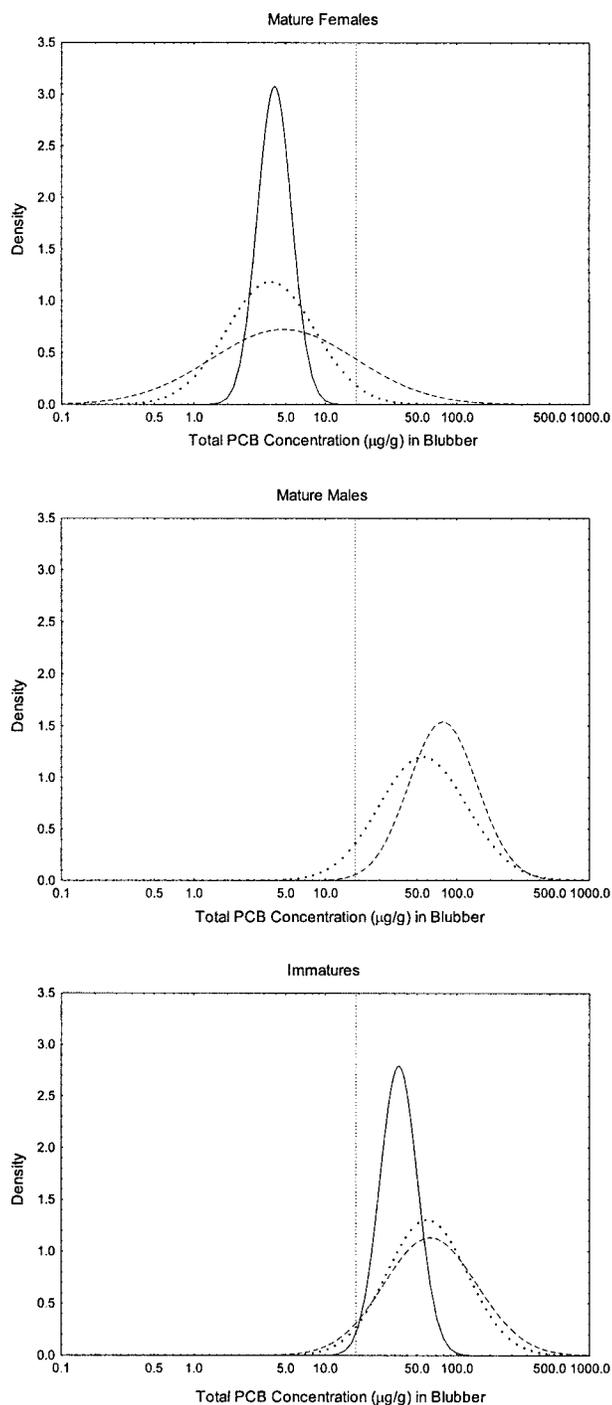


Fig. 2. Distributions of total polychlorinated biphenyl (PCB) concentration for different age/gender classes. Solid line represents Beaufort (NC, USA) population, dashed line represents Matagorda Bay (TX, USA) population, and dotted line represents Sarasota Bay (FL, USA) population. Distribution for Beaufort males is not shown because of small sample size ($n = 2$). The vertical dashed line is the threshold level for adverse effects as proposed by Kannan et al. [45].

from studies of dolphins or even other cetaceans would be preferable, but this is simply not realistic. However, risk assessment efforts should not be abandoned just because species-specific data are lacking but must proceed with the available information and be updated as new information becomes available. In this regard, a Bayesian analysis approach would lend itself well for estimating parameters for the applied concen-

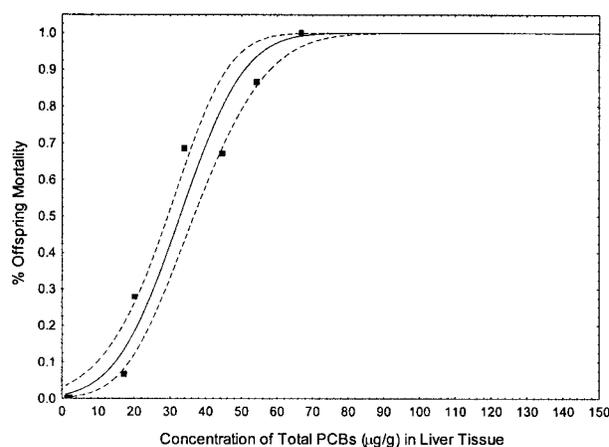


Fig. 3. Concentration-response curve with 95% fiducial limits for relationship between offspring mortality and polychlorinated biphenyl (PCB) concentration in liver tissue. Filled squares indicate original data points.

tration-response function and could be easily updated as toxicological data for additional species become available. Research into the underlying mechanisms of PCB reproductive toxicity is certainly to be encouraged and may ultimately provide a stronger basis for more quantitative interspecies extrapolation models in the future.

Our analysis addresses solely the risk associated with PCB exposure, although other organochlorine contaminants were measured in the tissues of the dolphins. We specifically chose to quantify adverse effects related to PCB exposure because the weight of evidence from experimental and epidemiological studies strongly supports the supposition that PCBs are a potential reproductive hazard to the studied populations. It is common to find PCB concentrations significantly correlated with concentrations of other persistent and ubiquitous contaminants, such as chlordane or DDT. These pesticides also may impair reproduction or infant development [74–76] and therefore might be expected to influence the risk of reproductive failure if their concentrations are significant. Given that additive or even synergistic effects may exist that are related to these cocontaminants, the estimates of risk presented here should be considered as lower bounds. Furthermore, if the concentration of some contaminant other than PCBs is uncharacteristically high for a given population of dolphins, the possibility of a significantly increased risk unaccounted for by our model should be investigated.

We have based our analysis on total PCB concentration in tissue, inferring that this single value can represent an indi-

Table 3. Expected risk of reproductive failure. Immature class represents females that are undergoing parturition for the first time. Confidence intervals were computed from 2.5th and 97.5th percentiles of 50,000 Monte Carlo simulations

Population	Age class	Expected risk	95% confidence interval
Beaufort (NC, USA)	Immature	0.60	0.23–0.82
	Mature	0.02	0.01–0.54
Matagorda Bay (TX, USA)	Immature	0.78	0.46–0.89
	Mature	0.10	0.02–0.55
Sarasota (FL, USA)	Immature	0.79	0.44–0.89
	Mature	0.03	0.01–0.48

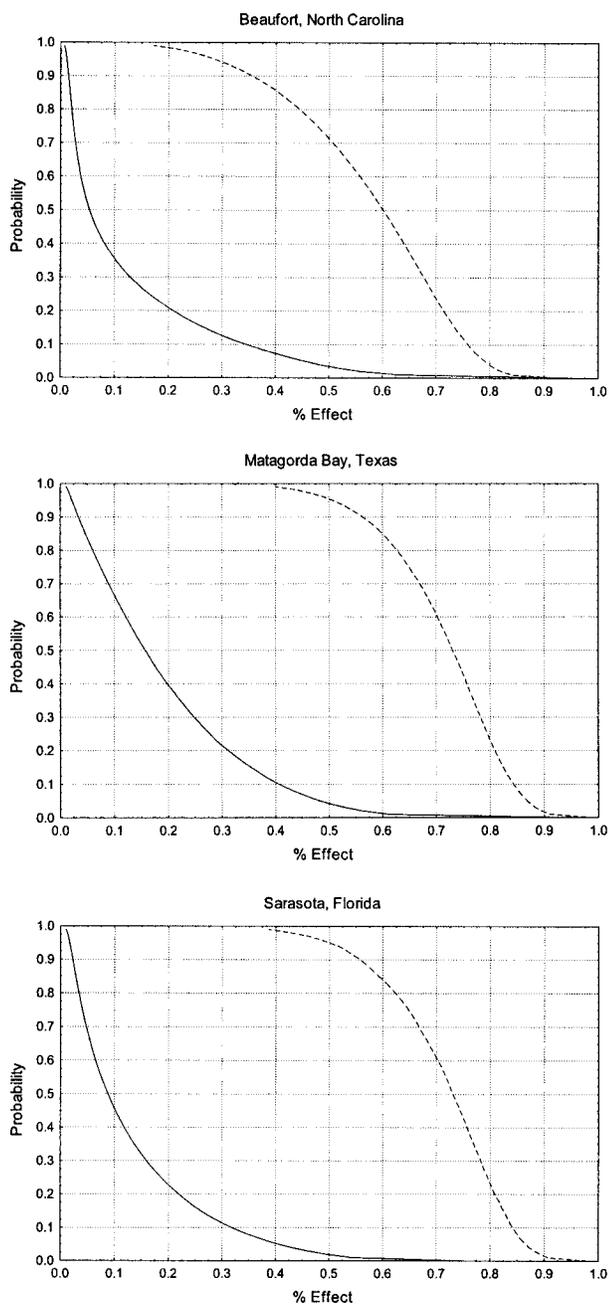


Fig. 4. Risk functions for female dolphins from each of the studied populations. Solid line represents risk function for mature age class (assumed multiparous), dashed line represents risk function for the immature age class (assumed primiparous).

vidual's PCB exposure. In fact, the exposure is not from a single chemical but from a complex mixture of chemicals with a range of toxic potencies. An alternative and possibly more accurate approach for estimating exposure and estimating a concentration response relationship can be based on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalence (TEQs) using toxic equivalency factors. Unfortunately, we were unable to perform a comparable analysis using the TEQ approach because of the lack of reported congener-specific data from experimental studies and the limited set of individual congeners analyzed from the dolphins' blubber. We have since expanded our analytical capabilities to quantify concentrations of additional congeners, and the TEQ approach will be investigated for fu-

ture efforts. It should be noted, however, that a shortcoming of the TEQ approach is that it may underestimate risk by ignoring the adverse effects of *ortho*-substituted nonplanar PCB congeners that do not interact with the AhR but elicit other non-dioxin-like effects (for discussion, see [77]). To be conservative, analyses using both approaches, that is, quantification of exposure using total PCBs and TEQs, should be performed and compared.

Finally, some concern exists that the dolphins sampled from the Beaufort and Matagorda Bay populations were survivors of one or more epizootic events and may not be representative of the PCB exposures in the populations as a whole. In particular, the blubber samples from Matagorda Bay were taken only months following the epizootic event. If the event were even indirectly related to the animals' contaminant exposure, then the dolphins that perished likely would have exhibited higher tissue concentrations than those that survived. If this were the case, then the calculated reproductive risk for this population would be an underestimate.

CONCLUSIONS

Determining population-level impacts that would be expected to result over time from our calculated reproductive risks would require stock-specific analysis, integrating information of population dynamics, age structure, and possible density-dependent or adaptive mechanisms. Although this is an important area for future research, the current findings in themselves provide enough information to warrant reevaluation of our current conservation and management practices. The analysis of risks for these populations indicates that reproduction, primarily in the primiparous females, is likely being severely impacted by chronic exposure to PCBs. The increased risk of reproductive failure for females giving birth to their first offspring will effectively increase the average age at first birth. A formal population model is not necessary to deduce that raising the age of first birth will likely impact the future growth potential or even stability of a population. At the very least, the PCB-related risk of reduced fecundity will affect the recovery of a population following reduction because of anthropogenic or environmental stresses, such as the recurrent epizootics that have been observed in the past several decades [78].

A primary intent of the congressional leaders who fashioned the Marine Mammal Protection Act was that a conservative bias be built into the legislation and that, in the face of lacking knowledge, a cautious approach be taken for the protection of these important species [79]. Nonetheless, current management plans under the Marine Mammal Protection Act do not consider the indirect anthropogenic stressors, such as contaminant pollution, when calculating human-induced mortality limits (or potential biological removal as termed under the Marine Mammal Protection Act [34]). The current management scheme to calculate mortality limits incorporates a mechanism to account for uncertainty in abundance estimates (using minimum stock size estimates) and a recovery factor to account for additional uncertainties related to biases in key parameters, such as maximum growth rate. Adjustment of the maximum growth rate, or at least application of a lower recovery factor, would seem appropriate for marine mammal populations that are highly exposed to PCBs and other POCs, putting them at high risk for reduced reproductive capacity.

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Table 4. Polychlorinated biphenyl (PCB) tissue concentrations associated with reproductive impairment in various mammalian species

Species	Study type	PCB tissue concn.	Observed reproductive effect	Reference
Mink	Experimental	44.6 µg/g lipid (liver); 2.19 µg/g wet wt (liver)	Impaired reproduction and increased kit mortality	[51]
Mink	Experimental	1.57 µg/g wet wt (liver)	Impaired reproduction and increased kit mortality (first generation)	[52]
Rhesus monkey	Experimental	32.69 µg/g wet wt (adipose)	Resorption, abortion, stillbirth for three out of eight pregnancies	[25]
Sow	Experimental	4.1–19.8 µg/g wet wt (adipose)	Fetal death, stillbirth	[19]
Baltic seal	Correlational/field observation	56–77 µg/g lipid (blubber)	Declining population; pregnant females = 56 µg/g, nonpregnant females = 77 µg/g	[23]
Baltic seal	Correlational/field observation	73–110 µg/g lipid (blubber)	Pathological changes in uteri; pregnant = 73 µg/g, nonpregnant with normal uteri = 89 µg/g, nonpregnant with stenosis/occlusions = 110 µg/g	[24]
California sea lion	Correlational/field observation	17.1–112 µg/g wet wt (blubber); 1.32–5.74 µg/g wet wt (liver)	Premature births; females with full-term pups = 17.1 µg/g, females with premature pups = 112 µg/g	[22]

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