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# Opportunities for using Navy marine mammals to explore associations between organochlorine contaminants and unfavorable effects on reproduction

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**Abstract**

The Department of Defense (DoD) has a unique marine mammal program maintained by the US Navy that includes the largest force of bottlenose dolphins, *Tursiops truncatus*, worldwide. In recent years, this population of cetaceans that lives in netted open water enclosures in San Diego Bay has been monitored for levels of organochlorine (OC) contaminants in blubber, blood and milk. Data generated from these studies have afforded insight into the fate and possible effects of OC contaminants in marine mammals. We now report preliminary findings on the effects of maternal OC exposure on pregnancy outcome. Blubber OC levels were compared between females whose calves survived beyond 6 months and females whose calves were stillborn or died within 12 days of birth. The mean concentration of ΣDDT was more than 3 times as high among dolphins whose calves died as that among dolphins whose calves survived beyond 6 months ($P = 0.002$). Mean ΣPCB was more than 2.5 times higher in females whose calves did not survive ($P = 0.076$). This population is a logical sentinel for the assessment of environmentally mediated disease. Biological tissues and fluids can be sampled on a regular basis from the dolphins for accumulation of tissue residues, facilitated by conditioned husbandry behaviors. These trained behaviors help preclude possible alterations in health measures resulting from capture stress. Animals' diets can be monitored for contaminant levels. With these data, the expertise and facilities available at the Navy laboratory and in collaboration with other experts in the field, controlled studies can be designed to monitor and assess dietary exposure, measurable immune and neurologic responses and assess reproductive and transgenerational effects of contaminants. Biomarkers can be developed to relate the health of individual animals relative to contaminant exposures. Such investigations of natural exposure and response scenarios are a logical adjunct to traditional laboratory toxicity studies. 

*Keywords:* DoD; Navy; Dolphin; *Tursiops truncatus*; Bottlenose dolphin; Organochlorines; PCB; DDT; Reproduction; Blubber; Blood; Milk; Lactation; Sentinel; Environmental contaminant; Biomarkers; Pollution

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## 1. Introduction

Marine mammals, such as dolphins, are likely targets for the accumulation and effects of ocean contaminants such as organochlorines (OCs). Their lipid-rich blubber, required for nutrition, buoyancy and insulation, is an ideal repository for these lipophillic contaminants (Jensen, 1989; Cockroft et al., 1990). In 1978 Risebrough stated, 'Research undertaken to date indicates that no marine mammal anywhere in the world is presently without a body burden of a variety of synthetic organic compounds which did not exist in the environment prior to their creation by man (Risebrough, 1978, pp. 1)'. The most inert OCs may remain in the blubber throughout the relatively long lives of marine mammals (Tanabe et al., 1984; Birnbaum, 1985; Calambokidis and Barlow, 1991). However, in times of physiological challenge such as illness, extreme temperature, nutritional compromise or pregnancy and lactation, OCs may be metabolized and circulated throughout the body by the bloodstream (Matthews et al., 1984; Humphrey, 1988; Alloway and Ayres, 1993; Aguilar and Borrell, 1994b). In many cases, it is the metabolites, not the parent compounds that have the greatest toxic effect (McFarland et al., 1986; Eisler, 1990).

OCs are transferred from one generation to the next mainly through lactation, not gestation (Fukushima and Kawai, 1981; Tanabe et al., 1982; Subramanian et al., 1988). Suckling offspring are at critical stages of growth and development (Beckman et al., 1999) and are, therefore, particularly sensitive to the effects of xenobiotic toxins that may be passed on from their mothers via lipid-rich milk (Holden and Marsden, 1967; Addison and Brodie, 1977, 1987; Cockroft et al., 1989). In cetaceans, maternal OC transfer is considered to be significant, especially for the first-born calf. Cockcoft and associates (1989) suggested that by the end of the first complete reproductive cycle, a bottlenose dolphin (*Tursiops truncatus*) transfers approximately 80% of her maternal body burden to her first-born calf. Fukushima and Kawai (1981) suggested that the first-born of striped dolphin females (*Stenella coeruleoalba*) receive 4 times more OCs than subsequent siblings, with more than 90% of the OC load transferred via lactation.

Marine mammals occur at high tropic levels in oceans worldwide. In recent years, reports of the frequency of epidemics and the emergence of new marine mammal diseases appear to be increasing (Harvell et al., 1999), resulting in unprecedented numbers of stranding reports and mass mortalities (Aguilar and Raga, 1993; Gerber et al., 1984; Marine Mammal Commission, 1999). Investigations into these events have often revealed high levels of OCs in tissues collected from the stranded animals (Geraci, 1989; Kuehl et al., 1991; Kannan et al., 1993; Aguilar and Borrell, 1994b; Kuehl and Haebler, 1995). Possible links of OC levels to pathology findings (Bergman and Olsson, 1985; Andersson and Wartanian, 1992; De Guise et al., 1994; Helle, 1980; Munson et al., 1998) and reproductive problems in marine mammals (DeLong et al. 1973; Helle et al., 1976a,b; Jensen et al., 1979; Reijnders, 1986; Béland et al., 1993; Kavlock et al., 1996) have added to the growing concern that OCs may be having a serious impact on marine mammal populations worldwide (Tanabe, 1992; Colborn and Smolen, 1996).

Globally, stranded animals have contributed a wealth of information on levels and patterns of contaminant levels in tissues (see Aguilar and Borrell, 1997). However, data from stranded animals are often incomplete. As pointed out by Aguilar and Borrell (1994b), there are problems in relying solely on data collected from stranded, diseased or moribund animals. The animals may not be representative of the entire population, there is usually a lack of relevant biological information making interpretation of data difficult and any sample decomposition can significantly affect analysis results (Borrell and Aguilar, 1990). Additionally, we know little of the biochemical consequences of measured contaminant levels in marine mammals (Addison, 1989).

One way to more accurately ascertain contaminant exposure and the effects of contaminant exposure on live marine mammals is to look at biomarkers (Aguilar and Borrell, 1994b; Fossi, 1994; Fossi et al., 1997; Gauthier et al., 1999). Because some biomarkers can have contaminant-

specific responses, they can be valuable for elucidating cause and effect relationships (Muir et al., 1999).

Experts attending a 1998 international workshop on marine mammals and persistent ocean contaminants pointed out the need for multidisciplinary studies conducted on both wild and captive marine mammals to determine the significance of OCs on marine mammal health and population dynamics (Marine Mammal Commission, 1999). They agreed that the ideal model species for these investigations were the California sea lion (*Zalophus californianus*), the harbor seal (*Phoca vitulina*), the bottlenose dolphin and the white whale or beluga (*Delphinapterus leucas*). These species have been studied in the wild and are well represented in marine mammal facilities where they have been studied extensively. Although the listed species are not endangered, they are good representatives of marine mammal species that are endangered. A recent census of zoos, aquaria and marine zoological parks in the United States and Canada (Andrews et al., 1997) reported that three of the model genera, *Tursiops*, *Zalophus* and *Phoca*, represented 78% of the animals at these facilities. Additionally, 70% of the *Zalophus*, 56% of the *Phoca* and 43% of the *Tursiops* in these collections were captive-born.

Of all the marine mammal models suggested for use by the Marine Mammal Commission (1999) for contaminant studies, more is known about the medicine, pathology and biology of *Tursiops* than perhaps any other species (Ridgway, 1968; Leatherwood and Reeves, 1990; Wells and Scott, 1999 for reviews). This species has a global distribution in warm and temperate water, which facilitates comparison of tissue levels from different populations of a single species. Additionally, *Tursiops* has both onshore and offshore populations that can be distinguished hematologically and morphologically (Duffield et al., 1983) allowing for comparison of levels of OCs in coastal and pelagic ecosystems. The most intensive long-term catch and release study of any wild marine mammal population in the world has been conducted on a population of *Tursiops* since 1970 (Scott et al., 1990).

Biomarkers from samples collected from animals with known health and reproductive histories should yield valuable data on long-term chronic exposure and transgenerational effects of contaminants. However, few wild populations of marine mammals are the focus of long-term monitoring programs and regulations often prohibit disturbing young or mothers with young. In addition, collecting blood samples from animals that have recently eaten could affect the blood concentrations of the compounds just as can mobilization of body fat as a result of disease (Matthews et al., 1984; Humphrey, 1988; Jensen and Slorach, 1991). The use of collection animals bypasses many of these obstacles because feeding schedules can accommodate sample collection and long-term health assessment data and reproductive histories are available. Reijnders (1988), pp 98) stated, 'Even more than before, marine mammals in captivity should be used to obtain a set of reference data to interpret values obtained from animals expected to be affected by contaminants'.

The largest collection of *Tursiops* is maintained by the Navy for the Department of Defense (DoD). Animals from this program are deployed worldwide in support of Navy missions and for Fleet exercises. Past deployments include Vietnam in 1970–1971 and the Persian Gulf in 1987–1988. When not on deployment, animals work from netted enclosures in San Diego Bay. Animals that do not deploy are available for various research and breeding programs. These animals have been conditioned for husbandry behaviors such as blood collection, milk collection and ultrasound imaging (Kamolnick et al., 1994; Ridgway et al., 1995). These voluntary behaviors facilitate the collection of samples and data and reduce the possibility of artifacts that may result from capture procedures.

Since its inception in 1959, the Navy's Marine Mammal Program has conducted important pioneering research on marine mammals including hydrodynamics, sonar, deep diving physiology, neurophysiology, hearing and sound production, behavior and reproductive physiology (LaPuzza, 1998). They also have been on the forefront in the development of improved techniques for diag-

nosis and treatment of health problems. In response to environmental concerns, Navy scientists have led the way toward beginning to understand the effects of sound pollution on marine mammals and have begun to explore the effects of ocean contaminants, specifically OCs, on these animals as well.

The Navy has a breeding program to provide a source of dolphins to replace animals that have completed their term of duty and are retired from the force. Because it has been suggested that OCs may be linked to reproductive problems in marine mammals (Jensen et al., 1979; Reijnders, 1986, 1994; Addison, 1989; Brouwer et al., 1989; Béland et al., 1993; Marine Mammal Commission 1999) it is important to assess any impact they may have on the success of the Navy's breeding program. Offspring can receive significant exposure to OCs through lactation (Addison and Brodie, 1977, 1987; Fukushima and Kawai, 1981; Tanabe et al., 1981; Jacobson et al., 1984; Martineau et al., 1987; Aguilar and Borrell, 1994a). To investigate exposure levels of suckling calves, milk samples were collected at various times during the course of lactation from female *Tursiops* in the Navy's breeding program. These females had been conditioned to allow trainers to use a modified human breast pump to collect milk samples (Kamolnick et al., 1994). The milk samples were assessed for levels of DDE and PCBs. For one dolphin, samples were collected from day 94 through day 615 of lactation. The lipid-normalized results for this animal showed that the highest levels of PCB and DDE were found in the milk samples collected near the beginning of lactation and decreased by 69 and 82%, respectively by day 615 (Ridgway and Reddy, 1995). Milk was also collected from two other dolphins that had spontaneously begun to lactate in response to orphaned calves (Ridgway et al., 1995). This event suggests the potential of inducing lactation in non-pregnant females —human or otherwise — to reduce OC body burdens and perhaps enhance pregnancy success.

Navy dolphins were also used to determine the relationship between levels of OC contaminants in red blood cells (RBC) and blubber for use when blubber biopsies are not possible or where serial sampling is desired (Reddy et al., 1998). For this, paired pre-prandial blubber and blood samples were collected from 16 clinically healthy *Tursiops*. Residue levels were quantified for 10 PCB congeners and 18 chlorinated pesticides using GC/MS. Significant relationships ($P \leq 0.05$) were found by regression analysis for HCB, *trans*-nonachlor, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT, tDDT, and PCBs 52, 101, 118, 128, 138(+158), 153, 170 and 187. Statistically significant $r^2$ terms ranged from 0.95 (*p,p′*-DDD) to 0.36 (*trans*-nonachlor) with a median of 0.64.

Table 1
*Tursiops truncatus*: maternal ID, age, calf number, lifetime days of lactation, sex of calf and age of calf at death

| Animal ID | Age | Length (cm) | Weight (kg) | No. calf | Lifetime days of lactation | Sex of calf | Calf age at death |
|---|---|---|---|---|---|---|---|
| SEA | 14 | NC | 182 | 2 | 1278 | F | NA |
| JAS | 7 | NC | NC | 1 | 0 | M | NA |
| OPA | 20 | 241 | 179 | 4 | 2144 | F | NA |
| BRT | 37 | 247 | 193 | 2 | 180 | M | NA |
| MUU | 21 | 244 | 160 | 3 | 929 | M | NA |
| SAD | 14 | NC | 182 | 3 | 26 | F | 8 days |
| COR | 7 | NC | 182 | 1 | 0 | F | 6 days |
| POP | 26 | 278 | 250 | 3 | 2 | M | Miscarried |
| DAS | 36 | 263 | 217 | 3 | 732 | M | Stillborn |
| CIR | 20 | 269 | 199 | 1 | 4 | M | 2 days |
| APR | 15 | 248 | 175 | 1 | 0 | M | 12 days |
| SUS | 17 | 246 | 145 | 1 | 0 | M | 10 min |
| BOE | 22 | 240 | 175 | 1 | 0 | ? | Resorbed? |
| PUN | 13 | 231 | 171 | 1 | 0 | M | Stillborn |

Slopes of the resultant regression curves can be used to estimate levels of OCs in *Tursiops* blubber when levels have been determined in RBCs. Developing additional relationships between blubber and serum, plasma and whole blood would be helpful when OC levels are only available for these blood fractions.

This group of animals was also used to assess the effect of maternal blubber levels of OCs on pregnancy outcome.

## 2. Methods

### 2.1. Study animals

The subjects (Table 1) were 14 adult female *Tursiops truncatus,* ranging in age from 7 to 37 years. Ten of the 14 animals were part of the Navy's Marine Mammal Program in San Diego, California, the other four were maintained at Six Flags Marine World in Vallejo, California. Each of these dolphins calved between 4/94 and 8/99 at either the Navy's marine mammal facility or at Six Flags Marine World. Miscarried fetuses and calves that were stillborn or died within the first 12 days of life were considered unsuccessful pregnancy outcomes. All the others survived beyond 6 months and were, therefore, considered successful outcomes. Since being collected in the Gulf of Mexico or along the east Florida coast, all the animals had regular physical examinations and have extensive records of reproductive and medical histories.

### 2.2. Sample collection

Blubber was opportunistically collected by biopsy from 12 of the subjects prior to parturition. In one case, it was not possible to collect a sample until 4 days post-partum. In another case, pregnancy was confirmed by ultrasound, but the fetus was either resorbed or miscarried so there is no parturition date. For the 13 animals for which a delivery date was available, the blubber was collected an average of 311 days prior to parturition (range 1514 pre- to 4 days post-partum). The collection site was approximately 10-cm lateral to the anterior insertion of the dorsal fin. The epidermis was anesthetized topically using liquid nitrogen or a refrigerant, dichlorotetrafluoroethane and the 0.2–0.5-gm blubber biopsy was collected with a modified 6-mm disposable biopsy punch (Miltex Instrument Company, Lake Success, NY). To ensure that the refrigerant would not affect analysis results, it was tested on control samples of blubber. No difference was found between the treated and untreated samples.

### 2.3. Analytical techniques

The methods for residue analysis are similar to those described by Reddy et al. (1998). Gas chromatography mass spectrometry (GC/MS) was used on all of the samples to determine levels of alpha-HCH, beta-HCH, gamma-HCH, heptachlor, *cis*-nonachlor, heptachlor epoxide, *cis*-chlordane, *trans*-nonachlor, HCB, mirex, dieldrin, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDT and *p,p′*-DDT. Additionally, 10 PCB congeners were chosen for analysis based on their reported prevalence and abundance in marine mammal tissues from previous studies (cf. Gaskin et al., 1983; Duinker et al., 1989; Kuehl et al., 1991; Muir et al., 1992; Schantz et al., 1993; de Kock et al., 1994; Falandysz et al., 1994; Kuiken et al., 1994; Hummert et al., 1995; Marsili and Focardi, 1995; Salata et al., 1995), potential for toxicity and their ease of separation using microsamples and GC/MS. The list of congeners was: IUPAC nos. (Ballschmiter and Zell, 1980) 52; 101; 105; 118; 128; 138 (co-eluted with 158); 153; 170; 180; and 187. To ensure quality control and inter-laboratory comparison, blanks and standards prepared using the National Institute of Standards and Technology (NIST) SRM 1945 whale blubber standard were run with each lot. Percent lipid was determined for each sample.

The Kilmogorov–Smirnov test was used to test normality and equal variance was tested with the Levene Median test. If normality and equal variance tests passed, then the differences between means were determined with an unpaired *t*-test. If either test failed, as it did for PCB 105, *o,p′*-DDE, *o,p′*-DDT and *p,p′*-DDT, a Mann–Whit-

ney Rank Sum test was used. In all cases, alpha = 0.05.

## 3. Results

All results were lipid normalized. The concentrations of PCBs are shown in Table 2a and concentrations of the chlorinated pesticides, alpha-HCH, beta-HCH, gamma-HCH, heptachlor, *cis*-nonachlor, heptachlor epoxide, *cis*-chlordane, *trans*-nonachlor, HCB, mirex, dieldrin, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDT and *p,p′*-DDT, are shown in Table 2b. To determine the effects of maternal OC exposure on pregnancy outcome, blubber OC levels were compared for those females whose calves survived beyond 6 months and whose calves were stillborn or died within 12 days of birth. Analysis of results comparing the OC contaminant load in the tissues of the two groups of females are presented in Table 3. The mean concentration of the sum of the six isomers and metabolites of DDT (*o,p′* and *p,p′* forms of DDD, DDE and DDT) was more

Table 2
Maternal *Tursiops truncatus* blubber concentrations of (a) PCB congeners and (b) chlorinated hydrocarbons (mg/kg, lipid wt.)

| (a) ID | PCB 52 | PCB 101 | PCB 118 | PCB 153 | PCB 105 | PCB 138 | PCB 187 | PCB 128 | PCB 170 | PCB 180 | *t*PCB |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Females whose calves survived beyond 6 months | | | | | | | | | | | |
| SEA | 0.095 | 0.095 | 0.046 | 0.660 | 0.095 | 0.491 | 0.680 | 0.041 | 0.281 | 0.561 | 3.04 |
| JAS | 0.174 | 0.292 | 0.266 | 1.437 | 0.045 | 1.245 | 0.639 | 0.143 | 0.260 | 0.617 | 5.12 |
| OPA | 0.029 | 0.076 | 0.025 | 0.084 | 0.022 | 0.070 | 0.051 | 0.018 | 0.018 | 0.029 | 0.42 |
| BRT | 0.187 | 0.364 | 0.411 | 3.843 | 0.166 | 3.750 | 1.660 | 0.373 | 0.866 | 2.299 | 13.9 |
| MUU | 0.073 | 0.173 | 0.195 | 1.647 | 0.065 | 1.548 | 0.839 | 0.196 | 0.375 | 0.852 | 5.96 |
| Females whose calves died within 12 days | | | | | | | | | | | |
| SAD | 0.481 | 0.380 | 0.290 | 7.141 | 0.108 | 7.427 | 2.835 | 0.770 | 1.301 | 3.165 | 23.9 |
| COR | 0.465 | 0.421 | 0.341 | 7.590 | 0.151 | 6.141 | 2.767 | 0.625 | 1.193 | 3.127 | 22.8 |
| POP | 0.213 | 0.526 | 0.438 | 1.615 | 0.142 | 1.592 | 0.663 | 0.246 | 0.328 | 0.911 | 6.67 |
| DAS | 0.149 | 0.322 | 0.320 | 0.572 | 0.121 | 0.602 | 0.211 | 0.084 | 0.072 | 0.254 | 2.71 |
| CIR | 0.188 | 0.304 | 0.240 | 1.869 | 0.084 | 1.816 | 0.702 | 0.232 | 0.366 | 0.751 | 6.55 |
| APR | 0.620 | 0.790 | 1.010 | 8.620 | 0.460 | 6.940 | 2.120 | 0.870 | 0.990 | 2.700 | 25.1 |
| SUS | 0.243 | 0.384 | 0.270 | 5.837 | 0.129 | 4.977 | 2.562 | 0.765 | 1.179 | 2.645 | 19.0 |
| BOE | 0.173 | 0.247 | 0.234 | 1.237 | 0.077 | 1.139 | 0.517 | 0.156 | 0.224 | 0.512 | 4.52 |
| PUN | 0.242 | 0.407 | 0.373 | 5.481 | 0.143 | 4.595 | 2.386 | 0.623 | 1.065 | 2.417 | 17.7 |

| (b) ID | α-HCH | β-HCH | γ-HCH | Hepta-clor | *Cis*-nonachlor | Heptaclor epoxide | *Cis*-chlordane | *Trans*-nonachlor | HCB | Mirex | Diel-drin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Females whose calves survived beyond 6 months | | | | | | | | | | | |
| SEA | 0.123 | 0.095 | 0.095 | 0.095 | 0.095 | 0.095 | 0.095 | 0.615 | 0.116 | 0.180 | 0.320 |
| JAS | 0.100 | 0.122 | 0.045 | 0.045 | 0.261 | 0.131 | 0.145 | 2.297 | 0.125 | 0.125 | 0.255 |
| OPA | 0.110 | 0.141 | 0.141 | 0.032 | 0.025 | 0.141 | 0.055 | 0.103 | 0.056 | 0.054 | 0.141 |
| BRT | 0.075 | 0.027 | 0.027 | 0.027 | 0.171 | 0.121 | 0.105 | 2.179 | 0.089 | 0.162 | 0.148 |
| MUU | 0.088 | 0.149 | 0.026 | 0.026 | 0.108 | 0.040 | 0.067 | 1.202 | 0.044 | 0.135 | 0.065 |
| Females whose calves died within 12 days | | | | | | | | | | | |
| SAD | 0.092 | 0.202 | 0.024 | 0.023 | 0.231 | 0.105 | 0.095 | 4.771 | 0.130 | 0.317 | 0.175 |
| COR | 0.110 | 0.185 | 0.059 | 0.059 | 0.429 | 0.214 | 0.105 | 7.961 | 0.107 | 0.506 | 0.196 |
| POP | 0.003 | 0.003 | 0.003 | 0.003 | 0.133 | 0.069 | 0.125 | 0.963 | 0.139 | 0.031 | 0.081 |
| DAS | 0.057 | 0.165 | 0.057 | 0.057 | 0.132 | 0.082 | 0.097 | 1.271 | 0.180 | 0.017 | 0.138 |
| CIR | 0.127 | 0.135 | 0.087 | 0.032 | 0.285 | 0.187 | 0.137 | 2.126 | 0.199 | 0.075 | 0.435 |
| APR | 0.045 | 0.045 | 0.045 | 0.045 | 0.140 | 0.110 | 0.060 | 2.730 | 0.180 | 0.070 | 0.220 |
| SUS | 0.095 | 0.141 | 0.016 | 0.039 | 0.438 | 0.096 | 0.157 | 5.246 | 0.238 | 0.357 | 0.117 |
| BOE | 0.003 | 0.003 | 0.003 | 0.023 | 0.058 | 0.057 | 0.047 | 0.882 | 0.094 | 0.098 | 0.063 |
| PUN | 0.065 | 0.101 | 0.048 | 0.048 | 0.405 | 0.068 | 0.120 | 5.578 | 0.109 | 0.418 | 0.076 |

Table 2 (*Continued*)

| | *o,p′*-DDE | *p,p′*-DDE | *o,p′*-DDD | *p,p′*-DDD | *o,p′*-DDT | *p,p′*-DDT |
|---|---|---|---|---|---|---|
| Females whose calves survived beyond 6 months | | | | | | |
| SEA | 0.043 | 2.032 | 0.095 | 0.200 | 0.095 | 0.095 |
| JAS | 0.161 | 8.386 | 0.123 | 0.809 | 0.139 | 0.224 |
| OPA | 0.033 | 1.016 | 0.012 | 0.087 | 0.141 | 0.141 |
| BRT | 0.223 | 23.180 | 0.139 | 1.203 | 0.690 | 0.512 |
| MUU | 0.172 | 5.939 | 0.062 | 0.547 | 0.130 | 0.190 |
| Females whose calves died within 12 days | | | | | | |
| SAD | 0.836 | 32.020 | 0.169 | 1.527 | 0.910 | 0.379 |
| COR | 1.293 | 33.940 | 0.468 | 1.884 | 0.831 | 0.412 |
| POP | 0.265 | 36.070 | 0.096 | 1.088 | 0.167 | 0.390 |
| DAS | 2.669 | 21.060 | 0.164 | 1.034 | 0.180 | 0.383 |
| CIR | 0.321 | 14.470 | 0.390 | 1.219 | 0.106 | 0.032 |
| APR | 0.510 | 32.730 | 0.260 | 2.230 | 1.010 | 1.000 |
| SUS | 0.895 | 26.270 | 0.310 | 1.986 | 0.864 | 0.442 |
| BOE | 0.152 | 18.480 | 0.044 | 0.683 | 0.104 | 0.265 |
| PUN | 0.771 | 19.470 | 0.230 | 1.701 | 0.588 | 0.433 |

than 3 times as high among dolphins whose calves died as that among dolphins whose calves survived beyond 6 months. The difference between the two groups was statistically significant ($P = 0.002$). Statistically significant differences between the two groups of females were found for the combined *o,p′* and *p,p′* forms of DDD ($P = 0.006$) and DDE ($P = 0.002$), but not for the combined *o,p′* and *p,p′* forms of DDT. Although not statistically significant, the mean concentration of *o,p′*- and *p,p′*-DDT in the group of females whose calves had died was twice as high as in the group of females whose calves had survived beyond 6 months.

The findings for other chlorinated pesticides were less consistent. Of the remaining group of contaminants, only HCB showed a statistically significant difference between the two groups ($P = 0.020$). A borderline non-significant difference was found for *trans*-nonachlor ($P = 0.083$). For the remaining compounds, dieldrin, heptachlor, heptachlor epoxide, mirex, alpha-, beta- and gamma-HCH, *cis*-nonachlor and *cis*-chlordane, there was little evidence of a higher blubber concentration in females whose calves died.

The mean concentration of the sum of all 10 PCBs (Table 3) was approximately 2.5 times as high in females whose calves did not survive compared to those whose calves survived beyond 6 months ($P = 0.076$). Significant differences were also seen for individual congeners PCB 52 ($P = 0.030$), PCB 101 ($P = 0.021$), and PCB 128 ($P = 0.042$). In the group that lost their calves, 67% were the first calf born of the female, while first-born calves represented 20% of calves born of females that produced surviving calves.

## 4. Discussion

The mean lifetime number of calves for females that had surviving calves was almost 1.5 times greater than those that lost calves. However, in the group that lost their calves, six of the nine (67%) born were first calves. In the successful group, only one was a first calf (20%). In the group that produced surviving calves, the average lifetime days of lactation was 906 compared to 85 in the group that lost their calves. Because OCs from the blubber are metabolized and excreted through the milk during lactation, the levels in the blubber are most likely reduced with increased periods of lactation (Aguilar, 1984; Addison, 1989; Aguilar and Borrell, 1994a). Therefore, the increased lactation in this group could account, at least in part, for the lower tissue levels of OCs. The higher number of mortalities of first-born calves seen here does not reflect

Table 3
Mean ± S.D. concentrations of OCs in mg/kg lipid wt. basis in maternal blubber of *Tursiops truncatus*

| Contaminant | Live ($n = 5$) | | Dead ($n = 9$) | | Normality[a] | |
|---|---|---|---|---|---|---|
| | Mean | S.D. | Mean | S.D. | Pass *t*-test | Fail Mann–Whitney |
| PCB 52 | 0.111 | 0.067 | 0.308 | 0.169 | 0.030 | |
| PCB 101 | 0.200 | 0.125 | 0.420 | 0.160 | 0.021 | |
| PCB 105 | 0.079 | 0.056 | 0.157 | 0.116 | – | 0.110 |
| PCB 118 | 0.188 | 0.160 | 0.391 | 0.241 | 0.122 | |
| PCB 128 | 0.154 | 0.142 | 0.486 | 0.304 | 0.042 | |
| PCB 138 | 1.421 | 1.429 | 3.914 | 2.658 | 0.078 | |
| PCB 153 | 1.534 | 1.434 | 4.440 | 3.113 | 0.075 | |
| PCB 170 | 0.360 | 0.312 | 0.747 | 0.488 | 0.139 | |
| PCB 180 | 0.872 | 0.853 | 1.831 | 1.197 | 0.142 | |
| PCB 187 | 0.774 | 0.579 | 1.640 | 1.088 | 0.128 | |
| tPCB | 5.692 | 5.073 | 14.334 | 9.109 | 0.076 | |
| α-HCH | 0.099 | 0.019 | 0.066 | 0.044 | 0.142 | |
| β-HCH | 0.107 | 0.049 | 0.109 | 0.076 | 0.959 | |
| γ-HCH | 0.067 | 0.05 | 0.038 | 0.029 | 0.191 | |
| Heptachlor | 0.045 | 0.029 | 0.037 | 0.018 | 0.515 | |
| *Cis*-nonachlor | 0.132 | 0.089 | 0.250 | 0.145 | 0.127 | |
| Heptachlor epoxide | 0.106 | 0.040 | 0.110 | 0.055 | 0.886 | |
| *Cis*-chlordane | 0.093 | 0.035 | 0.105 | 0.035 | 0.560 | |
| *Trans*-nonachlor | 1.279 | 0.959 | 3.503 | 2.491 | 0.083 | |
| HCB | 0.086 | 0.036 | 0.153 | 0.049 | 0.020 | |
| Mirex | 0.131 | 0.048 | 0.210 | 0.188 | 0.383 | |
| Dieldrin | 0.186 | 0.101 | 0.167 | 0.115 | 0.764 | |
| *o*,*p*′-DDE | 0.126 | 0.084 | 0.857 | 0.770 | – | 0.011 |
| *p*,*p*′-DDE | 8.111 | 8.933 | 26.06 | 7.926 | 0.002 | |
| *o*,*p*′-DDD | 0.086 | 0.051 | 0.237 | 0.137 | 0.037 | |
| *p*,*p*′-DDD | 0.569 | 0.455 | 1.484 | 0.511 | 0.006 | |
| *o*,*p*′-DDT | 0.269 | 0.253 | 0.529 | 0.387 | – | 0.142 |
| *p*,*p*′-DDT | 0.233 | 0.164 | 0.415 | 0.254 | – | 0.182 |
| tDDT | 0.472 | 0.414 | 0.944 | 0.587 | 0.140 | |
| tDDE | 8.237 | 9.004 | 26.91 | 7.934 | 0.002 | |
| tDDD | 0.655 | 0.498 | 1.720 | 0.605 | 0.006 | |
| Sum DDT (6) | 9.363 | 9.873 | 29.58 | 8.585 | 0.002 | |

[a] Normality tested with Kolmogorov–Smirnov test.

*Tursiops* calf survivorship statistics calculated from data obtained from a recent survey of *Tursiops* breeding programs at marine mammal facilities (Joseph et al. 2000). Collectively, these data show that of 28 first calves born, 75% were still alive after 2 years. The percentage drops to 68.8% ($n = 32$) and 66.7% ($n = 24$) for second and third calves, respectively, and then increases to 81.1% ($n = 11$) for the fourth calf. Wells (1991) suggests that from his observations of wild *Tursiops* where dolphins often lose their first and sometimes second calves, reproductive success increases with the age and probably the experience of the mother. Further research is needed to increase the data set as well as assess whether or not there are differences in the level and style of maternal care between primiparous and multiparous dolphins. These data should help to differentiate between factors affecting the reproductive success of these animals and may show that multiple factors may be affecting calf survival. One could hypothesize that a dolphin that has not previously given birth may be at a disadvantage for successful calf rearing because of high storage levels of

contaminants as well as limited maternal experience.

These findings should be considered preliminary. Additional animals are needed to increase the sample size and explore the significance of these findings further and ideally incorporate reproductive biomarkers. In summary, based on a small number of observations, these preliminary findings suggest that maternal blubber OC levels correlate strongly with reproductive outcome in *Tursiops*.

Continued contaminant studies with the Navy's marine mammals may not only demonstrate the effects of OC contaminants on marine mammals, they may also lead to insights on human effects of OCs. Bottlenose dolphins and humans share many features of contaminant exposure such as long gestation, long nursing periods and few offspring. They both have relatively long life spans and may be similarly impacted by the bioaccumulation of contaminants. As mammals, both humans and marine mammals can receive a high initial dose of contaminants through consumption of maternally produced milk. While adult humans consume a wide variety of foods, children and dolphins have less of a variety, with a resultant increased potential risk from any single food. As a marine species eating a diet comprised mainly of fish, dolphins are exposed to some of the same mixtures of environmental contaminants as fish-eating humans at the same trophic level, however, the dolphins' diets can be more easily monitored for dietary exposure levels. Levels found in fish can be used to develop global distribution maps of contaminant levels found in food fish. Some human populations such as Eskimos and other native peoples actually use marine mammals as a food source and, therefore, are exposed to high levels of lipophillic contaminants sequestered in the tissues (Chan, 1998).

## 5. Conclusion

The full potential of using this unique population of animals for OC investigations has yet to be realized. Such investigations of natural exposure and response scenarios are a logical adjunct to traditional laboratory toxicity studies. The use of DoD dolphins in these investigations is a unique and valuable opportunity to provide basic insight into the relationships between contaminants, health and physiological processes. They allow for investigations of transgenerational effects of long-term, low dose exposure and also provide opportunities to develop and test biomarkers. Biomarker technology is needed for the detection and characterization of the effects of various environmental stressors on the cellular and molecular processes that regulate organismal health and well-being. The evaluation and validation of potentially useful biomarkers requires long-term serial sampling and correlations with clinical health, hematological parameters and contaminant burden. The use and support of the DoD population of dolphins in environmental contaminant studies should help to reduce the scientific uncertainty about the fate and effects of some potentially toxic contaminants and bring us closer to understanding their pathological impact on humans and dolphins alike.

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