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RAPID COMMUNICATION: ORGANOHALOGEN LEVELS IN HARBOR SEAL (*Phoca vitulina*) PUPS INCREASE WITH DURATION OF NURSING

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Maternal transfer of persistent marine contaminants to offspring via milk has been documented in marine mammals, but temporal dynamics of this phenomenon throughout the lactation period are poorly understood. Exposures to organohalogens were investigated in harbor seal pups admitted to a rehabilitation center in north central California during the lactation periods of 2001 and 2002. Ten congeners of PCBs, three congeners of PBDEs, and p,p′-DDE were quantified in whole blood samples. Levels of contaminants increased with admit date, assumed to correlate positively with pup age. This trend was significant when latitude of stranding site, body condition, and body length were included as variables in the model. Contaminant–admit date relationships appeared nonlinear (i.e., threshold or exponential), with greatest increases in contaminant concentrations during late lactation.

Persistent marine pollutants bioaccumulate in marine mammals and can induce a range of adverse effects. Exposure of newborns to such chemicals is of particular concern, as young animals are immunologically immature and most vulnerable to such effects (Beckmen et al., 2003; Hall et al., 2003; Jenssen et al., 2003). Harbor seals (*Phoca vitulina*) have relatively short and intense lactation periods during which time females reduce their food intake, while secreting extremely fatty milk to support pups during this vulnerable life stage. Lipid mobilization from blubber produces release of some of the maternal burden of lipophilic contaminants into milk, and this transfer of contaminants from mother to pup is an important factor in the distribution of organohalogen contaminants in marine mammals (Addison & Brodie, 1987; Pomeroy et al., 1996; Debier et al., 2003).

Harbor seals along the California coast (*P. v. richardsi*) pup during spring and early summer, with a lactation and weaning period of 3-6 weeks (Temte et al., 1991; Greig, 2002). Each year during this time, a number of pups that

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have been separated from their mothers are brought to The Marine Mammal Center (TMMC, Sausalito, CA) for rehabilitation; this provides a research opportunity to examine changes in contaminant levels in pups with varying degrees of exposure to maternal milk. In this pilot study, the relationship was investigated between pup age, which in the case of stranded pups corresponded to duration of nursing, and contaminant levels in pup blood. It was hypothesized that pups admitted earlier in the season nursed less and would therefore possess relatively low contaminant loads, whereas pups admitted later in the season would have nursed longer and were predicted to contain higher blood concentrations of lipophilic contaminants.

METHODS

Pups ($n = 17$) found stranded along the central/north central California coast (35.08–38.66 latitude) were admitted to TMMC from 12 March to 22 June of 2001 and 2002. Pup mass and standard length (SL) upon admission ranged 5.4–15 kg and 64–93 cm, respectively. Whole blood samples were drawn from the extradural venous sinus into sterile evacuated collection tubes containing ethylenediamine tetraacetic acid (EDTA, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ), transferred to glass vials with tetrafluoroethylene, (TFE)-lined screw caps, and stored frozen until analysis.

Target analytes included polychlorinated biphenyl (PCB) congeners (IUPAC numbers) 128, 153, 156, 167, 170, 180, 189, 195, 206, and 209; polybrominated diphenyl ether (PBDE) congeners 47, 99, and 153; and *p,p′*-dichlorodiphenyl dichloroethylene (DDE), the primary metabolite of the insecticide DDT. CBs 14, 65, and 166 were used as method surrogates and CBs 30 and 204 as internal standards. PCBs and DDE were purchased from AccuStandard, Inc. (New Haven, CT), and PBDEs were purchased from Cambridge Isotope Laboratories (Andover, MA). Methods for analytical chemistry and quality assurance were presented in detail elsewhere (Neale et al., 2005). Briefly, extraction (acetonitrile/hexane) and cleanup (Florisil) of organohalogen residues from 5 ml whole blood was followed by quantitative analysis via gas chromatography–electron capture detection (GC-ECD) (injection 100°C, 1.5 min; 15°C/min to 165°C; 20°C/min to 285°C; run time 114 min). Multilevel internal standard calibrations using a minimum of five standard concentrations were used as the basis for quantification.

The duration of nursing of stranded pups would ideally be best measured by pup age upon admission to TMMC, but this was unknown. Although body mass and SL previously were used to index age in pinniped pups, both parameters were likely to be poor indexes in our study. Body mass is expected to be highly variable in nonnormal pups, such as the stranded sample here, and SL is only weakly correlated with pup age during late lactation (Cottrell et al., 2002). Therefore admit date was used as an index of nursing duration, but SL was included as a variable in statistical models, to accommodate any additional contribution by this factor. In addition, because pups were collected across a modest latitudinal range over which some cline in peak pupping season might be expected, latitude of stranding site was also included in statistical models. Finally, an index of condition based on mass relative to SL (Neale et al., 2005) was included because contaminant concentrations in blood can vary with body condition/adiposity (Lydersen et al., 2002).

Analyte concentrations were expressed on a wet-weight (ng/g whole blood) basis. For each sample, the measured concentrations of individual congeners were totaled to obtain ΣPCB and ΣPBDE values. Concentrations of *p,p′*-DDE, ΣPCB, and ΣPBDE were transformed [log(x + 1)] to approximate normality prior to statistical analysis. Statistical analyses of the relationships between contaminants (ΣPBDE, ΣPCB, and *p,p′*-DDE) in pup blood and independent variables (admit date, SL, latitude, and condition index) were performed in SYSTAT (version 9, SPSS, Chicago, IL) via multiple regression with backward stepwise elimination of nonsignificant variables.

RESULTS

Blood concentration (ng/g) ranges were 0.07–3.02 for ΣPBDE, 0.07–6.62 for ΣPCB, and <0.01–27.87 for DDE. Concentrations of all contaminant classes increased with increasing admit date (Figure 1). Duration of nursing, as indexed by admit date, explained more than 52% and 69% of the variation in ΣPBDE and ΣPCB, respectively, and—together with latitude of stranding site—explained >89% of the variation in DDE levels of pups. Additionally, although one individual had high leverage in the analysis (the pup with latest admit date and greatest concentrations of contaminants), identical analyses excluding this individual produced significant relationships between two of the three contaminants (ΣPCB and DDE) and admit date (p = .006 and p < .001, respectively). Individual congeners of PCBs and PBDEs followed the same trend; among congeners with quantifiable levels for ≥ 2 individuals, 6 of 7 were significantly positively correlated with admit date.

DISCUSSION

Although correlations in field data often can be misleading due to confounding unmeasured variables, it seems likely that findings in this study do indeed describe a relationship between contaminant levels and nursing duration. That latitude was not significantly related to admit date suggests that spatiotemporal sampling, although opportunistic, was sufficiently even. Further, the four individuals with highest contaminant levels came from four different counties, indicating that results were not confounded by location.

The functional relationships of (untransformed) contaminant concentrations to admit date were suggestive of threshold or exponential relationships. Such patterns were similar to those documented in a recent investigation of PCB dynamics in gray seal (*Halichoerus grypus*) milk (Debier et al., 2004). These observations support a hypothesis of PCB accumulation in a thinning maternal blubber layer throughout lactation, coupled with blubber retention

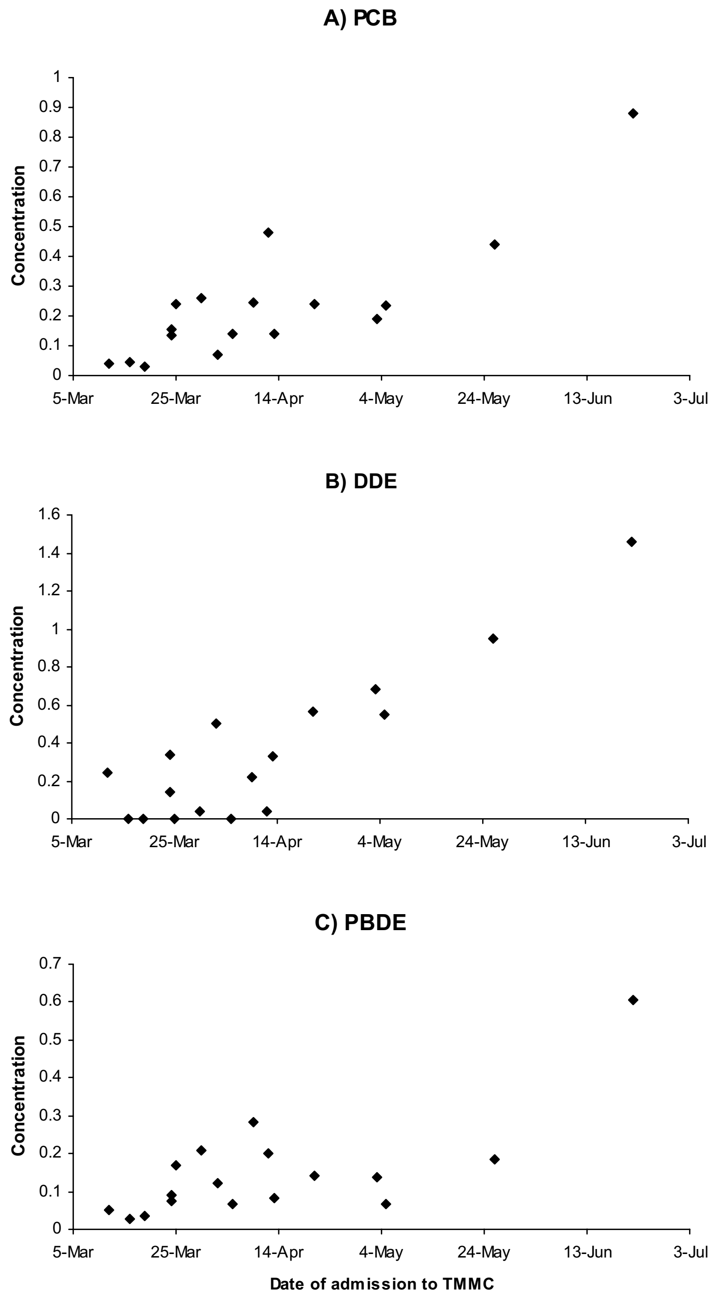

FIGURE 1. Organohalogen concentrations [log(ng/g wet weight + 1)] in whole blood of harbor seal pups ($n = 17$) increased with duration of nursing (as indicated by date of admission to The Marine Mammal Center). (A) ΣPCB: $R^2 = .693$, $p < .001$. (B) *p,p′*-DDE: $R^2 = .892$, $p < .001$. (C) ΣPBDE: $R^2 = .521$, $p = .001$. Statistics are from final multiple regression models in which only admit date was a significant factor of ΣPCB and ΣPCB; both admit date and latitude remained significant variables of *p,p′*-DDE.

thresholds for lipophilic compounds—phenomena resulting in increased maternal circulation of such chemicals during late lactation with corresponding rising levels in mother's milk and thus in circulating blood of pups.

Although not unexpected, the detection of a (statistically significant) relationship between contaminant loads in pups and admit date was noteworthy, given the many potentially confounding factors for which we were unable to account. These included (1) maternal effects, such as maternal age, health/nutrition status, prey preferences and feeding location, and exposure environment/history; (2) birth order effects; (3) the amount and lipid content of milk produced by individual mothers; and (4) the imperfect relationship between admit date and age. While accumulation of lipophilic contaminants via the food web and their transfer to offspring via lactation have been documented in pinnipeds, to our knowledge this is the first study to investigate temporal dynamics of this phenomenon in the harbor seal. Findings of this pilot study underscore the importance of lactation as a significant contaminant exposure pathway in young pinnipeds. Future study is needed to characterize the potential health effects of such exposures during this sensitive life stage.

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