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# Organochlorine contaminant concentrations in multiple tissues of free-ranging Steller sea lions (*Eumetopias jubatus*) in Alaska

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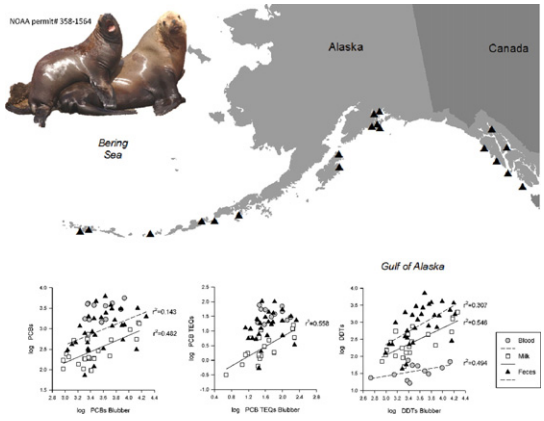
## HIGHLIGHTS

- Organochlorine contaminants are reported in blood, feces, milk, and blubber.
- The major PCB congeners were PCB101, PCB118, PCB138, and PCB153.
- ∑PCBs in blubber were related to concentrations in paired milk and feces.
- ∑DDTs in blubber were related to concentrations in paired milk, feces, and blood.
- ∑PCB TEQs in blubber was only related to ∑PCB TEQs in paired milk samples.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The relationships of selected organochlorine (OC) contaminants between blubber, blood, feces, and milk of young, free-ranging Steller sea lions (*Eumetopias jubatus*) were examined. Both between and within each tissue there was considerable individual variation. In spite of the variation, similar patterns were observed across the tissues for most of the selected PCB congeners. In all four tissues, the major PCB congeners were PCB101, PCB118, PCB138, and PCB153. The most prominent congener, both as a weight (ng/g lipid) and as a percentage of summed PCBs (∑PCBs), was PCB 153. Comparisons between paired tissues showed that ∑DDTs in blubber samples were related to concentrations in blood, feces, and milk. The ∑PCBs in blubber were related to concentrations in milk and fecal samples, though the relationship with feces was weak. Our findings show milk samples, in particular, are useful for assessing OCs in young sea lions. Blubber concentrations of PCB101, PCB118, and PCB138 were an order of magnitude higher than those in milk, supporting the biomagnification of these PCB

*Abbreviations:* OCs, organochlorine compounds; PCBs, polychlorinated biphenyls; DDTs, dichloro-diphenyl-trichloroethane; SSL, Steller sea lion; DPS, distinct population segments; EDTA, ethylenediaminetetraacetic acid; 1, 2, 3, 4-TCDD, 1, 2, 3, 4-tetrachlorodibenzo-p-dioxin; HPLC/PDA, high-performance liquid chromatography/photodiode array; HCB, hexachlorobenzene; UV, ultraviolet; LOQ, lower limit of quantitation; TEFs, toxic equivalent factors; TEQs, toxic equivalents.

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Blood
Blubber
Milk
Feces

congeners in SSL tissues. The findings indicate alternative tissues may be used as indicators of relative contaminant exposure in lieu of surgical blubber biopsy.



## 1. Introduction

Steller sea lions (*Eumetopias jubatus*, SSLs) range from northern Japan, along the North Pacific Ocean rim, through the Aleutian Islands and Bering Sea, extending along Alaska's southeastern coast and south to California (Burkanov and Loughlin, 2005; Loughlin et al., 1984). Within U.S. waters, SSLs are managed as two distinct population segments (DPS) with 144° west longitude (near Cape Suckling, Alaska) being the dividing boundary between the eastern and western DPS. The western DPS is currently listed as endangered under the U.S. Endangered Species Act (U.S. Federal Register 61:30772–30773) following a decline of more than 80% (Loughlin, 1998; Sease et al., 2001). The eastern DPS was previously listed as threatened until its recent delisting in 2013. The causes of the population decline and slow recovery have been the focus of continuing research and debate, with environmental contaminants including organochlorine compounds (OCs) being hypothesized as a contributing factor (Atkinson et al., 2008; Barron et al., 2003; National Marine Fisheries Service, 2008). However, the potential impact of OCs on the health and survival of SSLs have not been fully evaluated due, in part, to limited contaminant data currently available for this species (Alava et al., 2012; Lee et al., 1996; Myers et al., 2008; Zaleski et al., 2014).

OCs which include polychlorinated biphenyls (PCBs) and dichlorodiphenyl-trichloroethane (DDTs) are a class of ubiquitous lipophilic chemicals that persist in the environment (Beyer and Biziuk, 2009; Hellou et al., 2012; Muir and de Wit, 2010; Turusov et al., 2002). These compounds bioaccumulate and biomagnify within top-level predators and have been detected in the tissues of marine mammals world-wide (Braune et al., 2005; O'Shea, 1999; Law, 2014). The widespread use of these compounds has been associated with deleterious effects on the health and reproduction of wildlife (Bernanke and Köhler, 2009; Fisk et al., 2005; Mnif et al., 2011). In pinnipeds, reduced pup production, premature parturition, and altered immune function have been associated with exposure to OCs (DeLong et al., 1973; Gilmartin et al., 1976; Helle et al., 1976a, 1976b; Reijnders, 1986; Beckmen et al., 2003; De Swart et al., 1994, 1995a, 1995b, 1996; Ross et al., 1995, 1996). An altered immune system, potentially leading to increased disease susceptibility, has been postulated to have led to several unusual mortality events associated with viral infections, such as morbillivirus (Olsson et al., 1992, 1994). Further, higher concentrations of PCBs in blubber were associated with increased risk of carcinoma in California sea lions (*Zalophus californianus*) (Ylitalo et al., 2005a). These findings have led to an increased interest in assessing OCs exposure in free-ranging populations of pinnipeds.

Blubber is widely used for monitoring of OCs in marine mammals (Borrell et al., 2010; Greig et al., 2007; Gundersen et al., 2013; Kajiwara et al., 2001; Krahn et al., 2009; Peterson et al., 2014; Waugh et al., 2014); however, collecting sufficient blubber for analyses and tissue archive can be difficult. Thus, alternative matrices such as blood, feces, and milk, which can be obtained in less-invasive manners has been advocated for, especially for threatened or endangered populations (Boon et al., 1994; Fossi et al., 1997; Fossi and Marsili, 1997; Jenssen et al., 1994; Lundin et al., 2015). Further, it has been suggested that blood may be a better indicator of OC contaminant exposure risks than blubber (Boon et al., 1994; Ross et al., 2003; Myers and Atkinson, 2012), as blood is the tissue reflecting the actual exposure of bioavailable OC contaminants to the targets of their adverse physiologic effects: the immune, reproductive, and endocrine systems. Alternative matrices would support repeated sampling in longitudinal studies to investigate the dynamics of OCs between tissue stores, mother–pup pairs, and the impact of contaminants on the health of individuals and populations. In the current study, concentrations of OCs, including selected PCB congeners, DDTs and DDT metabolites, and hexachlorobenzene (HCB) were measured in blood, feces, and milk for comparisons with paired blubber samples collected from free-ranging pups and juvenile SSLs.

## 2. Materials and methods

### 2.1. Animals and sample collection

Free-ranging SSLs (N = 53) were captured, sampled, and released in Alaskan waters between 1998 and 2003 by personnel from the Alaska Department of Fish & Game (ADF&G) and the National Marine Mammal Laboratory of the Alaska Fisheries Science Center, National Marine Fisheries Service (NOAA Fisheries). Capture locations (N = 19) were on or near rookeries and haul outs from Lowrie Island (54.51 N 133.31 W) in the southeastern panhandle through the Gulf of Alaska, west to Adak Island (51.6459 N 176.9841 W) in the Central Aleutian Islands (Fig. 1). Sea lions were either captured and handled on land with hoop-nets or captured underwater using divers, and transferred to a research vessel for measurements and sampling as described by Fadely et al. (2005) and Raum-Suryan et al. (2004). Ages were estimated based on time of year, tooth eruption, and tooth measurements using the method of King et al. (2007), which is accurate through 2 years of age. Sea lions handled in the present study ranged in age from 1.5 to 35 months. The age of one SSL (35 months) was based on the seasonal stable isotope signature in a whisker (Rea et al., 2015).

Physical examinations and sample collection occurred under isoflurane anesthesia (Heath et al., 1997). All samples were kept chilled until returned to the ship and frozen at −20 °C until further processing and analysis. Blood samples (N = 13) were collected using standard aseptic techniques via venipuncture of a hind flipper vein (21 G butterfly catheter) or caudal gluteal plexus (18 to 20 G 1.5–2.5 in. needle) directly into polypropylene evacuated blood tubes (Vacutainer® Becton Dickinson, Franklin Lakes, NJ, USA) containing potassium ethylenediaminetetraacetic acid (EDTA). Whole blood was mixed with potassium EDTA by gentle inversion. Blubber samples (N = 48) were collected from a sterile surgical biopsy site 3–4 cm cranial and lateral to the femoral joint. The selected site was clipped of hair, scrubbed in triplicate with a 10% povidone-iodine (Betadine®) surgical scrub and rinsed with isopropyl alcohol-soaked gauze pads. The site was draped and a skin incision made with a #10 scalpel. A 6 mm biopsy punch was introduced down to the blubber/muscle interface and the blubber was extracted from the incision within the punch on withdrawal or by manual withdrawal using sterile forceps. Extracted blubber samples did not include any skin or muscle and were placed on a dry Teflon® sheet pre-cleaned with acetone. After folding in the edges of the Teflon®, the sample was sealed in a polyethylene bag (Whirl-pak®). Fecal samples (N = 33) were collected *per rectum* with acetone-rinsed, alcohol-disinfected sponge forceps and transferred to acetone-rinsed sheets of aluminum foil or Teflon®. If the animal defecated during the handling procedure, a fecal sample was collected in a plastic bag and subsequently sub-sampled and transferred to aluminum foil or Teflon® pre-rinsed with acetone. The foil or Teflon® was folded over the sample and then sealed in a polyethylene bag (Whirl-pak®). Milk samples (N = 21) were collected by orogastric lavage using a foal stomach tube and drenching syringe to create suction sufficient to aspirate no more than 100 mL of fluid stomach contents. If the fluid had the color, odor, and consistency of milk, the sample was transferred to a glass scintillation vial pre-rinsed with acetone.