

**INVESTIGATIONS OF GAMMAHERPESVIRUS INFECTION,
EXPOSURE TO PERSISTENT ORGANIC POLLUTANTS AND
METASTATIC CARCINOMA
IN CALIFORNIA SEA LIONS (*Zalophus californianus*)**

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Introduction

An 18% prevalence of neoplasms, the highest to date in a pinniped population, was reported in California sea lions (*Zalophus californianus*) that stranded live along the central California coast¹. The predominant neoplasm was a poorly differentiated carcinoma, which may be of urogenital origin, and occurs in sexually mature animals of both sexes. A recent study has shown that the tumors of California sea lions that stranded along the central coast of California contained cytoplasmic and nuclear virions that typify a herpesvirus². The virus was classified as a gammaherpesvirus and was observed in tumors from carcinoma-affected sea lions. Another study determined that this gammaherpesvirus appears to be distinct from another phocid herpesvirus, phocine herpesvirus-2 and tentatively designated the California sea lion virus as otarine herpesvirus-1 (OthV-1)³. After establishment of a PCR specific for OthV-1, the authors found that viral DNA was present in all urogenital tumors of California sea lions examined.

Although the etiology of the neoplasm in California sea lions is unknown, environmental contaminants may play a role. Certain types of chemical contaminants can directly induce carcinogenesis through DNA damage as initiators or complete carcinogens (acting as both initiators and promoters). For example, epidemiological studies, as well as studies on laboratory animals, indicate that PCBs can act as tumor initiators through oxidative stress mechanism⁴. In addition, environmental contaminants may be indirectly linked to carcinogenesis by acting as promoters (e.g., increasing cell proliferation) or as immune suppressors by increasing susceptibility to infection by oncogenic viruses⁵. Previous studies have shown that California sea lions are exposed to various POPs^{6,7}. Some of the compounds (e.g., PCBs) measured in these animals are in the concentration ranges that are associated with impaired immune function in other species of marine mammals^{8,9}. Based on these findings, exposure to high levels of immunosuppressive chemical contaminants in conjunction with infection by an oncogenic virus could potentially increase the risk of cancer in California sea lions.

In the present study, concentrations of selected POPs in blubber of California sea lions of various age and sex classes were measured and compared to herpesviral status (positive or negative). These data are part of a broad, multidisciplinary study to examine various physiological,

biological and environmental factors that influence gamma herpesvirus infection and metastatic carcinoma in California sea lions.

Materials and Methods

Blubber samples of free-ranging California sea lions from San Miguel Island (premature pups, yearlings, juveniles, adult females) and Puget Sound, WA (adult males), as well as blubber from pregnant female /fetus sea lion pairs that died as a result of domoic acid toxicity were collected and analyzed for selected POPs, including dioxin-like PCB congeners and DDTs, by a rapid high-performance liquid chromatography photodiode array (HPLC/PDA) method¹⁰. Briefly, blubber (0.2-0.4g), hexane/pentane (1:1 v/v), sodium sulfate (5g) and a surrogate standard were homogenized and separated from interfering compounds (e.g., lipids, aromatic compounds) on a gravity flow cleanup column that contained neutral, basic and acidic silica gels eluted with hexane/methylene chloride. Prior to the cleanup step, a 1-mL aliquot of each sample extract was removed for lipid quantitation by thin layer chromatography with flame ionization detection (TLC/FID)¹¹. The dioxin-like congeners (PCBs 77, 105, 118, 126, 156, 157, 169, 189) were resolved from other selected PCBs (PCBs 101, 128, 138, 153, 170, 180, 190, 200) and POPs by HPLC on two Cosmosil PYE analytical columns, connected in series and cooled to 16°C. The congeners were measured by an ultraviolet (UV) photodiode array detector and were identified by comparing their UV spectra (200-310 nm) and retention times to those of reference standards in a library.

To determine the prevalence of gammaherpesvirus infection, vaginal or prepuccial mucosal swabs were collected from free-ranging California sea lions, as well as from animals that had recently died. We developed the ability to detect OthV-1 genome (infection) in DNA extracted from mucosal swabs by standard polymerase chain reaction (PCR) using OthV-1 primers specific for a 697 bp fragment of the DNA polymerase gene (For 5'TTACACTTCTACgTgATgg 3', and Rev 5'CA ATGATACTGG ACGAAGA) designed based on the published sequence (GenBank AF236050), and CSL interleukin-2 and/or mammalian ferritin as reporter (control) genes.

Results and Discussion

Mean (\pm SE) concentrations of percent lipid, total PCBs (tPCBs), summed DDTs (sumDDTs) and summed PCB TEQs (sumPCB TEQs) measured in the blubber of California sea lions are reported in Table 1. California sea lions are exposed to POPs throughout all life stages and the concentrations of POPs increase in sea lions of both sexes until sexual maturity. Males continue to accumulate these lipophilic contaminants throughout their lives. In contrast, concentrations of POPs in adult (reproductive) females decrease due to maternal transfer of these lipophilic compounds to offspring during gestation and lactation. DDTs were the most prevalent POPs measured in blubber of all sea lions except in adult males. The most prevalent POPs measured in adult males were PCBs. These differences in prevalences of POPs among the various age and sex classes of sea lions are most likely due to different foraging areas of adult males (California to British Columbia) compared to the foraging regions (mostly off the California coast) of sea lions from the other classes.

Blubber concentrations of tPCB TEQs measured in juvenile and adult male California sea lions are in the TEQ concentration range that is associated with immune dysfunction in harbor seals fed herring from the contaminated Baltic Sea^{8,9}. If these sea lions are immune suppressed due to contaminant exposure, they could be more susceptible to OthV-1 infection than animals that are not immunocompromised. Furthermore, epidemiological studies, as well as studies on laboratory animals, indicate that certain POPs (e.g., PCBs) can act as tumor initiators through oxidative stress

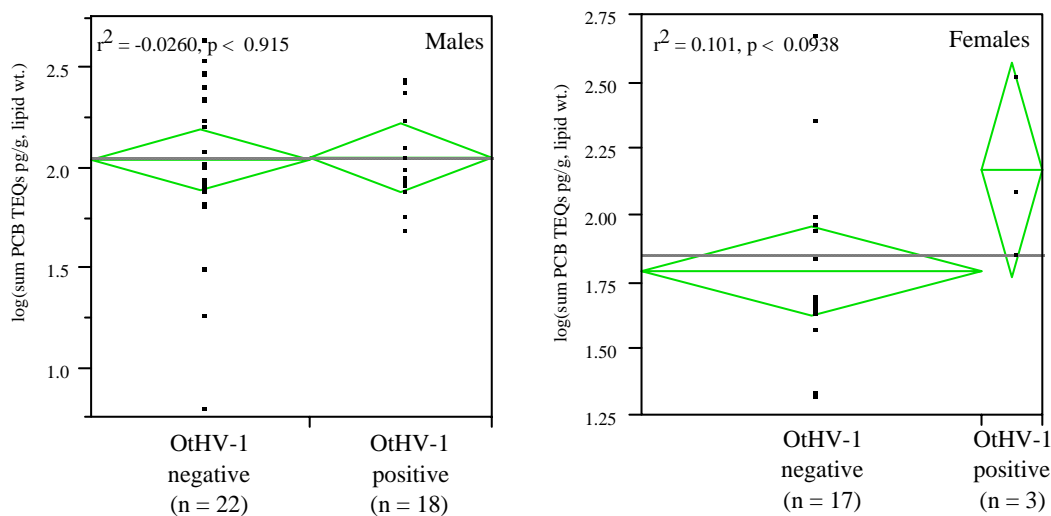
mechanism or tumor promoters (most likely through the Ah receptor and inhibiting intercellular communication)^{4,5}. Based on these findings, exposure to high levels of POPs, especially compounds that are associated with immune suppression as well as tumor promotion, in conjunction with infection by an oncogenic virus could potentially increase the risk of cancer in California sea lions.

We compared the POP blubber concentrations (log transformed) to presence of gammaherpesvirus (see Materials and Methods for detection description) in muscosal swabs of adult male (prepuccial) or female (vaginal) sea lions. For both male and female sea lions, we found no correlations with herpesvirus status and POP concentrations when based on wet weight. We found better correlations between OtHV-1 status and concentrations of tPCBs (data not shown) and sumPCB TEQs in adult females than the correlations between OtHV-1 status and blubber concentrations of these contaminants in adult male California sea lions (Figure 1), although these correlations were not statistically significant. It is interesting to note that many of male sea lions that were gammaherpesvirus negative had comparable or higher blubber concentrations of PCB TEQs than did the positive gammaherpesvirus female sea lions. These findings may be related to sex-related differences in susceptibility to OtHV-1 infection or to differences between the sexes in exposure to other potential carcinogens that have not been determined in this study. In contrast to tPCBs and sumPCB TEQs, we found no correlations between virus status and concentrations of sumDDTs (based on lipid weight) in either adult male or female sea lions. Our preliminary findings indicate that there may be a correlation between OtHV-1 status and concentrations of PCBs and PCB TEQs in female California sea lions, but due to the small sample size, no conclusions can be drawn. Further studies are warranted in determining the levels of contaminants and prevalence of OtHV-1 in free-ranging California sea lions as well as interactions between this gammaherpesvirus and exposure to chemical contaminants or additional anthropogenic or natural factors and how they may have a role in the etiopathogenesis of these tumors in California sea lions.

Table 1: Mean (\pm SE) concentrations (ng/g, lipid weight) of tPCBs, sumDDTs and sumPCB TEQs (pg/g, lipid weight) in blubber of California sea lions of various age and sex classes.

Age and sex class	Site	tPCBs	sumDDTs	sumPCB TEQs
Fetuses (n = 12)	TMMC	8,600 \pm 1,500	29,000 \pm 6,400	98 \pm 20
Pregnant females (n = 12)	TMMC	8,100 \pm 2,500	26,000 \pm 8,900	83 \pm 23
Premature pups (n = 4)	SMI	5,600 \pm 1,900	21,000 \pm 8,000	130 \pm 49
Yearlings (n = 3)	SMI	7,600 \pm 1,700	25,000 \pm 6,400	180 \pm 37
Juveniles (n = 12)	SMI	12,000 \pm 2,100	41,000 \pm 11,000	300 \pm 55
Adult females (n = 20)	SMI	5,300 \pm 1,200	14,000 \pm 4,100	100 \pm 27
Adult males (n = 40)	PS	55,000 \pm 8,800	30,000 \pm 3,600	800 \pm 120

Figure 1: Ranges of log(sumPCB TEQs) concentrations (based on lipid weight) in blubber of California sea lions based on OtHV status and sex.



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