PERSISTENT ORGANIC POLLUTANT CONCENTRATIONS IN SOUTHERN SEA OTTERS (*ENHYDRA LUTRIS NEREIS*): PATTERNS WITH RESPECT TO ENVIRONMENTAL RISK FACTORS AND MAJOR CAUSES OF MORTALITY

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Submitted to

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EXECUTIVE SUMMARY

A comprehensive study of persistent organic pollutants (POPs) was performed on 227 freshly dead wild southern sea otters (*Enhydra lutris nereis*) stranding between 2000 and 2005 along the California coast between San Francisco and Ventura. For each animal a complete necropsy was performed by the California Department of Fish and Game, providing detailed data on each otter's age class, sex and nutritional condition, as well as data on the primary and contributing causes of death. Consideration of these data, along with each otter's stranding location and liver POP concentrations permitted detailed statistical analyses of the spatial, environmental and demographic relationships with the detection of high POP concentrations in sea otters, as well as relationships between elevated liver POP concentrations and major causes of sea otter death.

This study addressed five major objectives, as follows:

1) Determine the types and concentrations of POP burdens occurring in the southern sea otter population.
One-hundred thirty-eight compounds were tested and 117 were detected in southern sea otter livers. All values were reported on a wet-weight basis. The highest average concentrations for POP groups were for dichlorodiphenyltrichloroethanes (DDTs; 635 ng/g), followed by polychlorinated biphenyls (PCBs; 177 ng/g) and polybrominated diphenylethers (PBDEs; 48.1 ng/g). The single analyte with the highest average concentration was p,p' DDE (614 ng/g), which was detected in all 227 samples. PCB 153 (30.3 ng/g) and PCB 138 (24.6 ng/g), also were detected in all 227 samples. The top five analytes were rounded out by PBDE 047 (23.1 ng/g), which was detected in 225 samples, and tributyltin (19.4 ng/g), which was detected in 97 samples.

2) Determine whether high POP tissue burdens are associated with regions characterized by large freshwater runoff and/or high levels of municipal wastewater input and high concentrations of human population.
Univariate statistical analyses indicated that several demographic factors were associated with high concentrations of POPs, which differed among major pollutant groups (e.g., pesticides, PCBs, butyltins, etc.). For most POPs, immature and subadult otters and animals with no or scant subcutaneous fat had the highest liver concentrations. Otters from areas characterized by moderate or high exposure to freshwater flows had significantly higher concentrations of DDD(o,p'), DDD(p,p'), DDE(o,p'), HCH delta, dibenzothiophene-C2 and PBDE 017 than otters that came from areas with low exposure to freshwater flows. Otters that came from areas with medium exposure to wastewater discharges had significantly higher concentrations of DDE(o,p'), dibenzothiphene-C2, PBDE 028, PBDE 066, PBDE 085 and PBDE 099 than did otters with low wastewater exposure. Otters with low wastewater exposure had higher concentrations of PCB 200 and PCB 209 than did otters with moderate wastewater exposure. Concentrations of some butyltins, acenaphthylene and PBDE 183 in sea otter livers varied with coastal human population density: In general, otters stranding in areas with coastal human population densities >3,000 persons/mile^2 had higher liver POP concentrations. While univariate analyses indicated that several POPs were higher in sea otters with moderate exposure to wastewater, compared to those with low exposure to wastewater, multiple-regression analyses

revealed that moderate exposure to wastewater was not a significant positive variable in any models explaining high concentrations of POPs. Nevertheless, multiple regression analyses revealed a negative association between moderate exposure to wastewater and high concentrations of PCBs in sea otter livers, although no stranded sea otters were recovered from areas with high exposure to wastewater.. Otters exposed to moderate or high levels of freshwater runoff were more likely to have high liver concentrations of some DDTs, chlordanes and butyltin compounds. Otters stranding near areas of moderate coastal human population density (3,000–6,000 persons/ mi^2) were more likely to have high concentrations of dieldrin, several chlordanes, dibutyltin and PCBs.

3) Investigate associations between high tissue levels of POPs in sea otters and POP concentrations detected in shellfish (e.g., mussels or clams) previously collected and tested from the same or adjacent areas.

Based on Musselwatch surveys, high POP concentrations in mussels were spatially associated with detection of high concentrations of the following POPs in livers of stranded sea otters from the same local areas: sumDDT, sumChlordanes, sumPCBs, and dieldrin. These findings were significant in multifactor models that also accounted for liver POP variation due to sea otter age class, gender, and body condition. These results suggest that local POP concentrations in filter-feeding mussels may be predictive of liver POP burdens of sumDDT, sumChlordanes, sumPCBs, and dieldrin for threatened southern sea otters stranding in the same areas.

4) Investigate associations between high tissue concentrations of POPs and specific causes of mortality in sea otters, including major sources of infectious disease.

A few POPs were significantly correlated with the presence of specific infectious diseases, as well as traumatic death, including C1-dibenzothiopene, PCB 056, cis-chlordane, oxychlordane and PBDE 028. Striking differences were found with regard to associations between increased liver concentrations of particular POPs and the detection of significant bacterial infections, acanthocephalan peritonitis and systemic protozoal infections in stranded otters. These preliminary findings suggest that some POPs may contribute to the risk of sea otter death due to specific infectious agents, such as bacteria, acanthocephalans and protozoa, as well as death due to trauma. However, when all causes of infectious disease were pooled and other factors such as sea otter sex, age, stranding area and nutritional condition were accounted for in multivariate models, no summed or individual POPs were found to correlate with an increased or decreased risk of otter death due to infectious disease. Based on these preliminary analyses, several demographic, spatial and environmental risk factors, including otter age class, nutritional condition, stranding location and proximity to moderate to high coastal discharge of surface runoff or moderate municipal wastewater were found to be as or more important predictive factors of sea otter death due to infectious or traumatic disease than were high concentrations of POPs in liver tissue. However, due to the complexity of POP effects in living animals and the focal nature of exposure to some pollutants, it is possible that some effects were present, but were not detectable using the current study design. Additional studies are in progress to analyze the data using techniques that more closely match those of prior studies, where positive associations between sea otter death due to infectious disease and elevated tissue POP burdens were reported.

5) Describe trends for POP concentrations in sea otters that can be used to evaluate the efficacy of management actions taken to limit contamination of nearshore waters by POPs from agricultural, urban and harbor sources.

a. This study provides a rigorous baseline for POP concentrations in sea otters in California. Future analyses can be done to determine trends and whether management actions taken to reduce discharges of contaminants to the marine environment have been effective. Comparison of the results from this study to those from previous investigations indicates that concentrations of DDTs in sea otter livers have declined since 1970. Additional analyses are in progress to examine this trend.

b. This study has illustrated the difficulty of pinpointing the deleterious effects of POPs on southern sea otter health, especially when the data are fully stratified to account for the effects of age, gender, nutritional location and stranding location, as was done here. We have clearly demonstrated the importance of including multivariate statistical approaches in addition to univariate statistical approaches for POP studies in marine species due to the complicated interactions among animal sex, age, nutritional condition, location, tissue POP burdens and cause of death. Future analyses using the data derived from this study will facilitate more accurate comparisons with previous studies, which focused on analyzing relationships between POP concentrations and the ultimate cause of death, rather than the involvement of infectious diseases or trauma in the overall findings from necropsy, histopathology and other diagnostic tests, as was done in the current study. Until these additional analyses are completed, no final conclusions should be inferred regarding associations between liver POP burdens in sea otters and major causes of death in southern sea otters.

c. Improved management and enforcement should be considered for coastal locations where high POP concentrations were detected in sea otter livers and shellfish, both to help reduce POP contamination in threatened sea otters and their prey and also to minimize POP exposure for humans who utilize these areas for recreation and harvest and/or consume local marine invertebrates.

ABSTRACT
Here we present the results of a multi-year epidemiological study focused on investigating demographic, environmental and spatial risk factors for exposure of sea otters to persistent organic pollutants (POPs) along coastal California. An additional objective was to examine the associations between elevated liver POP burdens and major primary and contributing causes of sea otter death. In addition to being a federally protected threatened species, California's southern sea otters (*Enhydra lutris nereis*) also have the potential to serve as ideal upper trophic level sentinels of nearshore pollution by anthropogenic waste, including chemicals and pathogens; Sea otters possess 4 unique biological traits that distinguish them from all other marine mammals in California: 1) They have comparatively small home ranges, 2) They have a comparatively high resting metabolic rate, requiring consumption of high volumes of marine foods, 3) They prey heavily on filter-feeding invertebrates, which can concentrate chemical and biological pollutants when present in local water and sediments, and 4) They live and feed along the immediate shoreline, which places them in close contact with plumes of anthropogenic wastes entering the ocean. Thus, southern sea otters are an ideal species to monitor to assess long-term trends in POP deposition to the local environment, as well as to monitor the environmental effects of planned and ongoing mitigation and control measures. In the present study, samples from 227 wild sea otters stranding between 2000 and 2005 along the California coast were tested for the presence of most major classes of POPs, including PCBs, PBDEs, PAHs, organochlorine pesticides and organotins. Potential contributors to the risk of POP exposure that were considered in the various statistical models included sea otter sex, age class, nutritional condition and stranding location, as well as the proximity of each stranding location to major points of coastal freshwater runoff, municipal wastewater discharge or dense coastal human populations. Of 138 compounds examined, the vast majority (117) were detected in livers of stranded, freshly dead southern sea otters. DDTs had the highest mean liver concentrations of all major contaminant groups, followed by PCBs and PBDEs. The POP analyte found at the highest levels in liver was p,p' DDE, with a mean of 614 ng/g wet weight, followed by PCB 153 (30.3 ng/g wet weight) and PCB 138 (24.6 ng/g wet weight). When overall population means were compared between this and prior studies, hepatic concentrations of DDTs appear to have declined significantly in southern sea otter livers since 1970. Sea otter age class, sex, and nutritional condition were significant risk factors for POP detection in sea otter livers. . In general, immature sea otters had the highest liver concentrations of POPs, emaciated animals had higher POP levels than animals with abundant body fat, and the effect of gender varied, with females having higher liver POP concentrations than males for some POPs that were significantly affected by gender. With regard to sea otter mortality, the top 3 most important findings at necropsy were considered in all statistical models, because some common disease processes, including trauma and infectious disease are often present concurrently in southern sea otters. When fully stratified for age, gender and stranding location, no significant positive or negative disease correlations were found for total butyltins, PBDEs, PCBs, DDTs or any other pollutant groups, when examined with respect to the top 3 most important lesions present at necropsy. However, liver concentrations of some POP analytes were significantly correlated with the presence of specific infectious diseases, as well as traumatic death, including C1-dibenzothiopene, PCB 056, cis-chlordane, oxychlordane and PBDE 028. Striking differences were found with regard to associations between increased liver concentrations of particular POPs and sea otter death due to bacteria, acanthocephalans and protozoa. However, when all causes of infectious disease were pooled for analysis, no summed or individual POPs were found to

correlate with an increased or decreased risk of sea otter death due to infectious disease, when other risk factors such as sea otter sex, age, stranding area and nutritional condition were accounted for in multivariate models. Based on these preliminary analyses, several demographic, spatial and environmental risk factors, including nutritional condition, stranding location and sea otter proximity to moderate to high coastal discharges of surface runoff and municipal wastewater were found to be as or even more important predictive factors of sea otter death due to infectious or traumatic disease than was POP detection at high concentrations in liver. This is the first study to screen tissues from a large number of freshly dead southern sea otters selected without bias to the stranding location or cause of death, and to fully stratify the resulting data for potential demographic, spatial and environmental risk factors that could confound the perceived risk of POP exposure for sea otter death due to infectious disease. Studies are currently in progress to re-examine these data in the context of each otter's primary cause of death and to more directly compare our research findings with those from other marine mammal studies.

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INTRODUCTION AND BACKGROUND

The southern sea otter (*Enhydra lutris nereis*) is a federally listed threatened species found only along the central coast of California. At the turn of the 19th century, these animals were thought to be extinct due to massive overharvest for the fur trade. However, a small remnant population of 40 to 50 sea otters was discovered along the remote and rugged Big Sur coast in central California in the early 1900's and this remnant population has served as the nucleus for species recovery. The southern, or California sea otter was first designated as a protected species in 1911 after its re-discovery. In 1977, southern sea otters were designated as a federally protected threatened species due to concerns regarding their small population size and limited geographic range, rendering this population highly susceptible to extinction from a single, large-scale environmental catastrophe, such as an oil spill.

Now in 2007, despite nearly a century of protection, the southern sea otter population has failed to increase its numbers sufficiently to merit de-listing, and repeated episodes of population decline have added to these concerns. Even under the best of conditions, southern sea otters have never increased at levels commensurate with their northern counterparts in Washington, Canada, Alaska and Russia; under optimal conditions, these other populations have increased by 15 to 20% each year, while rates of increase for southern sea otters have never exceeded 6%, and were often much lower, or were negative (Reidman and Estes, 1990). Until recent years, underlying causes of this slow population increase, repeated declines and overall failure of southern sea otters to recover were unclear. Comparisons with other sea otter populations regarding reproductive success have revealed similar levels of fecundity; thus impaired reproductive success was not the cause.

However, when systematic carcass recovery programs were implemented, starting in the late 1970's, a high proportion of the population was recovered dead along the coast each year, prompting concerns about impacts of mortality on southern sea otter recovery. At present, ≥200 southern sea otter carcasses are recovered each year throughout their range, and because a great deal of this habitat is remote and rocky, it is certain that numerous dead otters are missed. Given that the current southern sea otter population is approximately 3,026 individuals, this suggests that ≥ 10% of these animals die each year. At present, there is universal consensus within the research community that one major factor limiting southern sea otter recovery is continuing high mortality. While the underlying causes of these deaths may be hotly debated, it is clear that southern sea otters suffer an extraordinary level of mortality attributed to infectious agents, including bacteria, parasites and fungi. Between 40% (Thomas & Cole, 1996) and 60% (Kreuder et al., 2003) of southern sea otter deaths have infectious agents at the core of the cascade of events leading to death. In addition, otters dying with significant trauma commonly have concurrent infections that likely enhanced their risk of traumatic death (Kreuder et al., 2003). In the present study, over 78% (179/ 227) of random-source, freshly dead sea otters received for necropsy between 2000 and 2005 had infectious processes as a primary or major contributing cause of death.

As the causes of southern sea otter morbidity and mortality have become better understood, a high proportion are now known or suspected to have strong ties to land-based pollution. Potential sources of illness stem from exposure to both biological pollution (e.g., terrestrial-origin bacteria and parasites), and chemical pollution, in the form of discharges of runoff or wastewater containing a vast array of anthropogenic compounds. These biological and chemical

pollutants appear to be both directly and indirectly impacting southern sea otters and jeopardizing their recovery.

However, the specific mechanisms of disease induction are often subtle and complex, particularly with respect to long-term exposure and bioaccumulation of persistent organic pollutants (POPs). Studies of laboratory animals and accidental exposures in humans have documented a wide range of impacts of POPs on host immunity, reproduction, cognition, mentation and endocrine function, among many others. However, the direct translation of those findings to animal morbidity and mortality in natural ecosystems is far more difficult and complex, because of a lack of strict controls for comparison, and difficulties accounting for the wide range of synergistic effects documented for various POPs. In addition, related, but structurally distinct analytes in a single POP class often exert diverse and sometimes antagonistic effects on living systems at the metabolic, cellular and subcellular levels. Additionally, these pollutants are not static in nature, but instead undergo a range of metabolic and environmental changes that may convert the original compounds to more toxic or environmentally persistent forms. These pollutants may enter the body transplacentally, via lactation, via postweaning foraging activity and through other routes, including transdermal absorbtion and inhalation, exerting their effects on the unborn fetus, neonates and older animals. Finally, many POPs are strongly lipophilic and have the ability to both biomagnify in aquatic systems and bioaccumulate within individuals.

Despite this broad and diverse array of effects, it is essential to monitor and investigate the potential impacts that POPs may be exerting on southern sea otter recovery. Past studies have revealed high levels of some POPs and other substances, especially butyltins, PCBs, organochlorines and DDTs in southern sea otter tissues (Shaw, 1971; Kannan et al., 1998; Nakata et al., 1998; Bacon et al., 1999; Kannan et al., 2004; Kannan et al. 2006a; 2006b) (Table 24). Potential relationships between contaminant concentrations and sea otter death due to infectious disease have been reported (Kannan et al., 1998; Nakata et al., 1998).

Some unique attributes of sea otter biology make them ideal sentinels for monitoring environmental pollution: As the world's smallest marine mammal, sea otters have developed unique adaptations to the cold-water environment in which they live. The first is the development of a luxurious fur coat , which is the densest of any living mammal at over 1 million hairs per square inch (Williams et al., 1992). It is this beautiful pelage that almost led to the sea otter's demise through strong demand for clothing between the 18^{th} and 19^{th} centuries. An additional adaptation that sea otters have developed for coping with the high energetic demands of their cold-water habitat is an extraordinarily high metabolic rate. Each otter must consume $\geq$ 25% of their own weight in prey each day to maintain condition; this requirement increases substantially during pregnancy and lactation (Reidman and Estes, 1992). Southern sea otters consume numerous species of marine and estuarine invertebrates, including mussels, clams, crabs, snails, cephalopods and worms; many of these invertebrates are detritivores or filter feeders that serve as highly efficient concentrators of both chemical and biological pollutants present in contaminated water (Fayer et al., 1998; Fayer et al., 1999; Graczyk et al., 1998; Graczyk et al., 1999; Arkush et al., 2003; Lindsay et al., 2004; Booj, 2002; Cornwall, 1995). In addition, sea otters are nearshore feeders, spending their entire lives along the coastal shoreline. Taken collectively, these attributes place sea otters directly in the path of terrestrial contaminant

discharges and ensures that they will be maximally exposed to any pollutants that are discharged into the nearshore marine environment.

There is ample reason to be concerned about these impacts that extends far beyond direct concerns for threatened southern sea otter recovery. In addition to serving as important environmental sentinels, these animals serve a critical role in their environment as keystone species, helping to maintain the complex, three-dimensional structure of the kelp forest through predation on kelp-grazing macroinvertebrates (Reidman and Estes, 1992). The kelp forest in turn provides ideal habitat for a wide range of marine wildlife, including invertebrates, fish and other marine mammals. The end result is a far richer nearshore marine environment than would exist if the kelp understory was not preserved. The kelp forest also provides direct benefits to humans by serving as a buffer to decrease wave-mediated shoreline erosion and as critical habitat for several fish, cephalopod and crab species favored by humans.

In addition, because sea otters consume many of the same prey species as humans, the detection of high levels of POPs and potential biological pollutants in sea otters provides additional impetus for monitoring and preserving this population. Based on results of recent studies (Miller et al., 2002b; Kreuder et al., 2003; Conrad et al., 2005; Miller et al., in press), southern sea otters may be the finest upper trophic level aquatic sentinel of environmental pollution ever discovered. For all of these reasons, the core recommendations made by the Southern Sea Otter Recovery team in 2003 include directives to: 1) prioritize analyses of tissues from southern sea otters for environmental contaminants; 2) determine sources of environmental contaminants present in sea otter prey and their habitat; and 3) evaluate causes of otter mortality relative to contaminant exposures (United States Fish and Wildlife Service, 2003). The current study is the first to directly address these core recovery objectives by using tissues and data from a large number of sea otters selected without bias to the location or cause of death, and by fully stratifying the resulting data for potential demographic, spatial and environmental risk factors, so that all factors that could contribute to the risk of both POP exposure and sea otter death are identified and accounted for. Because an enhanced risk for otters with high tissue POP levels dying with infectious disease was reported in previous studies and because of the widespread concern that southern sea otters could be suffering from POP-mediated immune suppression, we agreed that this issue merited large-scale, systematic epidemiological research. A multi-year research proposal was prepared and submitted to the Proposition 13 California State Water Resources Control Board funding initiative in January, 2002 to investigate this issue and project funding was awarded in October, 2002. The preliminary product of this multidisciplinary effort is this report, with additional analyses and publications to follow. This study has been a collaborative effort between the Central Coast Long-Term Environmental Assessment Network, the California Department of Fish and Game, Office of Spill Prevention and Response and the University of California at Davis, Wildlife Health Center.

OBJECTIVES

There are 5 specific objectives for this research:

1) Determine the types and concentrations of POP burdens occurring in the southern sea otter population.

2) Determine whether high POP tissue burdens are associated with regions characterized by large riverine inputs, agricultural runoff, street or surface urban runoff and/or high levels of municipal wastewater input.

3) Investigate associations between high tissue levels of POPs in sea otters and POP concentrations detected in shellfish (e.g., mussels or clams) previously collected and tested from the same or adjacent areas.

4) Investigate associations between high tissue concentrations of POPs and specific causes of mortality in sea otters, including major sources of infectious disease.

5) Describe trends for POP concentrations in sea otters that can be used to evaluate the efficacy of management actions taken to limit contamination of nearshore waters by POPs from agricultural, urban and harbor sources.

A REVIEW OF MAJOR CLASSES OF PERSISTENT ORGANIC POLLUTANTS EXAMINED IN THIS STUDY

PCBs

Polychlorinated biphenyls (PCBs) are anthropogenic persistent pollutants that are widespread in the environment, having been widely manufactured for over 70 years for various industrial uses, including capacitors, hydraulic oils and industrial lubricants, due to their chemical stability at high temperatures (Sanchez-Alonso et al., 2004). Over 200 possible PCB congeners exist, based on the number and position of chlorine atoms on the biphenyl rings; 135 different congeners have been detected in the environment (Sanchez-Alonso et al., 2004). The reactive chlorine content of PCB mixtures may be substantial, ranging from 18-68% of total molecular weight (WHO, 1993). All PCBs, but especially more highly chlorinated congeners are highly lipophilic, environmentally persistent and can be transported long distances in water, wind and sediments; as a result, food chain magnification and progressive PCB accumulation within individuals are worldwide concerns, and PCB utilization and disposal have been more tightly restricted for over 30 years. Even so, PCBs are now routinely detected in animals and humans residing in the remotest regions of the earth, including the arctic (Verreault et al., 2006; Wolkers et al., 2006). For coplanar PCBs (i.e., those for which the biphenyl rings are maintained in the same plane due to the placement of chlorine substitutions on the rings), the reported average intake in European populations is similar to the estimated tolerable dose (ATSDR, 2000), with a large proportion of the population exceeding these doses (Johanssen et al., 2006); subtle adverse effects of prenatal exposure on child development have been suggested at current levels (Johanssen et al., 2006).

Toxicity and specific effects of PCBs are structure-dependent: congeners with ≤ 1 chlorine substitution in the ortho position may assume a planar configuration, bind to the aryl

hydrocarbon (Ah) receptor and elicit dioxin-like activity; these are termed coplanar PCBs. Coplanar PCBs induce host metabolic enzyme activation in a pattern similar to administration of 3-methycholanthrene (Sanchez-Alonso et al., 2004). Some general toxic effects of exposure to coplanar PCBs, such as PCB 70, in humans and laboratory animals include chloracne and other skin lesions, immunotoxicity, inhibition of weight gain and reproductive toxicity. PCB 126, a coplanar congener, is one of the most toxic PCBs studied (Safe, 1994). With >1 chlorine substitution in the ortho position, the rotation of the rings can become sterically hindered and the dioxin-like activity disappears; these are termed nonplanar PCBs. Nonplanar PCBs induce host metabolic enzyme activation in a pattern similar to administration of Phenobarbital (Sanchez-Alonso et al., 2004). Non-coplanar PCBs, exemplified by PCB 153, are generally considered to be less toxic, but are generally detected in tissues at higher levels than are the dioxin-like PCBs. PCB 153 is one of the most abundant PCBs in human tissue, and is sometimes used as a biomarker for the total PCB burden (Johanssen et al., 2006). However, more recent studies suggest that nonplanar congeners such as PCB 153 can exert stronger effects on cell signaling pathways and can be more efficient at inducing apoptosis than some coplanar PCBs (Chen et al., 2006; Fernandez-Santiago et al., 2006).

PCBs were manufactured and utilized as mixtures of several congeners known as aroclors through the 1970's, when further manufacturing was banned or highly restricted (Ma and Sassoon, 2006); since that time levels of PCBs in the environment have decreased and food-based PCB exposure has been reduced (Lee et al., 2007). However, it is estimated that over 70% of the more than one million tons of PCBs that were manufactured are still in use (Ma and Sassoon, 2006). Arochlor mixtures are designated with a 4 digit number (e.g., arochlor 1248): The first pair of numbers indicates the dominant planar/ nonplanar designation of the mixture and the second pair denote the total chlorine content by percentage of total weight (e.g., 48% for the example above).

A wide range of cellular mechanisms have been suggested for toxic effects due to PCBs, including alteration in neurotransmitter levels (e.g., dopamine), induction of metabolic enzymes (e.g., CYP1A1), and perturbations in intracytoplasmic cell signaling and second messenger pathways via effects on protein kinase C, caspases, Bax, Bcl-2, calcium, calpains and cathepsins, ultimately leading to induction of cellular necrosis or apoptosis. Important target organs for PCB activity include the brain and pituitary, as well as a wide range of endocrine organs whose effects are moderated by the secretory products of the pituitary (Sanchez-Alonso et al., 2004; Johanssen et al., 2006). Other potential target tissues include immune cells (Keller et al., 2006; Lyche et al., 2006), gonads or reproductive tract (Ma and Sassoon, 2006), thyroids (Lee et al., 2007), splenocytes (Yoo et al., 1997), vascular endothelial cells (Slim et al. 2000), kidneys (Chen et al., 2006; Fernández-Santiago et al., 2006) and skin and dermis. Weak estrogenic effects are also noted in multiple studies (Arcaro et al., 1999; Hany et al., 1999; Lind et al., 1999; Ma and Sassoon, 2006). These impacts in exposed humans and laboratory animals have resulted in neurodevelopmental defects, memory deficits, alterations in sensory and cognitive function, persistent increases in motor activity and abnormal changes in behavior (Lee et al., 2007). Reproductive and developmental defects in fetuses of exposed animals have included increased embryonic death, delayed implantation, and abortion (Ma and Sassoon, 2006). Importantly, lactational transfer represents the primary route of PCB exposure for developing mammals, with <5% of PCB transfer during gestation and the majority occurring postnatally during lactation (Buck, 1996). In humans, enhanced susceptibility to infectious disease has been reported,

especially in children whose mothers were exposed to high levels of PCBs (Tryphonas, 1995). In marine mammals, potential toxic effects of PCBs reported in prior studies include endocrine disruption (Simms et al., 2000; Troisi and Mason, 2000), reproductive impairment (DeLong et al., 1973) and cancers (De Guise et al., 1994a; 199b). Both *in vivo* and *in vitro* studies suggest that organochlorines can be immunotoxic in marine mammals (Levin et al., 2007).

PBDEs

Polybrominated diphenylethers (PBDEs) are ubiquitous industrial compounds that are incorporated into a wide range of commercial and household products as flame retardants. Products that commonly contain these compounds include computers, fax machines and printers, as well as carpets, flooring and upholstery. Commercially-produced PBDEs are generally classified into 3 main groups: penta-, octa- and decabromodiphenyl ethers, so-named for the bromination pattern of the major constituent of each product (Schecter et al., 2003). Approximately 50,000 tons of PBDEs are produced each year worldwide (Schecter et al., 2003), with about 80% produced as deca-PBDEs, followed by penta- (12%) and octa-PBDEs (6%) (Schecter et al., 2003). Over 33,000 metric tons of PBDEs were marketed in the United States alone in 2001 (Schecter et al., 2003). Penta-PBDEs are more pliable and are often used as flame retardants in polyurethane foam used to upholster furniture, while hexa and deca-PBDEs are often mixed into harder plastic products at the time of manufacture, such as computers.

PBDEs, particularly penta-PBDEs are widely used in North America, especially the United States. Public concern over widespread application of these products is increasing due to several factors: 1) Their high production volumes and structural similarity to contaminants known to be toxic to humans, such as PCBs and 2) their demonstrated ability to bioaccumulate. Unlike levels for now-banned or strictly-controlled pollutants (such as PCBs) that are slowly decreasing over time in human tissues, several recent studies have demonstrated a rapid and alarming increase in PBDE concentrations in humans and wildlife, especially in the United States (Betts, 2002; de Wit, 2002; Solomon and Weiss, 2002; Darnerud, 2003; Watanabe and Sakai, 2003; Sjödin et al., 2004); 3) PBDEs have also been demonstrated to cause disease, including neurobehavioral, endocrine, and renal defects in laboratory animals (Darnerud, 2003).

Although the acute toxicity of PBDEs is generally low in laboratory animals (Darnerud, 2003), more chronic effects can be significant, especially with higher or continuous exposures (Darnerud, 2003). In addition, recent experiments have demonstrated that these negative impacts appear to correlate with the bromination pattern of the dominant congener: Although penta-PBDEs are commercially produced in lower quantities in some areas, these lower brominated congeners bioaccumulate to a greater degree in humans and animals than octa- and deca-PBDEs, and appear capable of causing adverse effects at lower doses. Repeated penta-PBDE exposure has been linked to impairment of neurobehavioral development and altered thyroid hormone homeostasis in rodents and humans (Schecter 2001, Darnerud, 2003). Exposure to octa-PBDEs was associated with fetal toxicity and teratogenicity in rats and rabbits, and deca-PBDE exposure was associated with altered thyroid, liver and kidney morphology in adult animals at high exposure levels, as well as induction of hepatocellular and thyroid adenomas and carcinomas (Darnerud, 2003). Other studies have demonstrated impairments in sexual development and androgenesis in rodents, especially males (Lilienthal et al., 2006).

Humans may be exposed to PBDEs, which are highly fat-soluble, through ingestion of food or dust, or via inhalation (Schecter et al., 2003). Heavy contamination of house dust with higher brominated PBDEs has been reported in some studies (Betts, 2003; Rudel et al., 2003; Sjodin et al., 2003; Watanabe et al., 2003), along with higher occupational exposure for computer technicians, computer repair personnel and those involved in manufacture of rubber (Sjödin et al., 1999; Thuresson et al., 2002; Thomsen et al., 2001; Darnerud, 2003). However, PBDEs are commonly detected in humans with no known occupational exposure (Darnerud, 2003), often at high levels. Fatty fish such as salmon appear to be efficient bioaccumlators and are thought to be a major source of human exposure (Voorspoels et al., 2006; Hayward et al., 2007). Other general human sources of PBDEs include biosolids prepared from sewage sludge used to fertilize fields. High levels of PBDEs have been found in human serum, adipose tissue and breast milk in several countries, especially the United States and Canada, (Darnerud, 2003). In Indiana, individual fetal blood concentrations did not differ significantly from corresponding maternal blood concentrations, demonstrating that fetal exposure to PBDEs begins prior to lactation (Mazdai et al., 2003). A sample of pooled breast milk from Texas and Colorado averaged 200 ppb total PBDEs, based on lipid weight (Papke et al., 2001). Total PBDE levels as high as 418 ppb have been detected in breast milk collected in Texas in recent years (Schecter et al., 2003). Penta-PBDE levels in serum and adipose tissue from women residing in the San Francisco Bay area in the late 1990s ranged from 5-510 ppb, with a median of 16.5 ppb (Petreas et al., 2003). PBDE levels detected in humans from European countries average 10 times lower than those found in North America, likely in part due to longstanding restrictions on PBDE marketing and use in EU countries (Schecter et al., 2003).

Recent studies have demonstrated that aquatic systems and biota are highly efficient at biomagnifying PBDEs (She et al., 2002; Voorspoels et al., 2006). In Canada, PBDE concentrations in human breast milk increased approximately 10-fold between 1992 and 2002 (Ryan et al., 2002). In contrast, a 100-fold increase in total PBDEs was detected in blubber from San Francisco Bay area harbor seals sampled over the same general time period (She et al. 2002).

DDTs

1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) was first synthesized in 1874 by Othmar Zeidler, a graduate student at the University of Strasbourg. DDT is not a naturally occurring chemical. In 1939 Paul Hermann Müller discovered the insecticidal properties of DDT and was awarded the Nobel Prize in Physiology or Medicine in 1948. DDT was widely used during world war II to control malaria and other vector-borne diseases and was used extensively as a pesticide after 1945. Between 1945 and 1972 approximately 1,350,000,000 pounds of DDT were used in the United States alone. Agricultural use in California was 1,164,699 pounds in 1970, 80,800 pounds in 1972, and less than 200 pounds per year from 1975 – 1980 (Mischke, 1985). In 1962, Rachel Carson's book "Silent Spring" was published and raised public awareness and concern regarding the extensive use of DDT and the potential negative impacts of DDT in the environment. In 1972, the US EPA banned DDT use in the United States except for use for emergency human health response, use on a few specific crops, and production for exportation. The Montrose Chemical Corporation factory in Torrance, California continued to produce DDT for export until 1982. This factory was a major source of DDT exposure that was later implicated in the sharp decline of brown pelicans, a federally listed threatened species. The Montrose factory discharged DDT into the Los Angeles County sewer system that emptied into the Pacific

Ocean off the Palos Verdes Peninsula. Over time, DDT breaks down to to form DDE through the loss of a chlorine. DDE interferes with avian eggshell formation, resulting in inadequate calcite deposition in eggshells. The end result is thin, brittle eggs that often break under the weight of adults attempting incubation (Davison, 1978). Eggshell thinning caused by DDE contamination was the main factor implicated in the decline of brown pelican populations breeding on the Channel Islands, just off the southern California coast (Blus, 1971). The decline of brown pelicans and their subsequent recovery after DDTs were banned in1972 is a classic example of the potential negative environmental impacts of DDT.

In addition to interference with eggshell formation, DDT and its metabolites exhibit other toxic effects in humans and animals, including neurotoxicity, hepatotoxicity, metabolic disruption, reproductive impairment and cancer. The following summary is condensed from the U.S. Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease Registry, 2002 Toxicological Profile for DDT, DDE, and DDD (http://www.atsdr.cdc.gov/tfacts35.pdf):

DDT affects the central nervous system by altering the opening and closing of cellular transmembrane sodium and potassium channels (Ecobichon, 1995; Narahashi and Haas, 1967). In the brain, this results in disruption of the normal activity of neuronal adenosine triphosphatase (ATPase) (Matsumura and Patil, 1969) and may also inhibit calcium ion transport in nerves (Matsumura 1985). These cellular alterations result sustained depolarization of nerve cell membranes and enhanced release of neurotransmitters, resulting in tremors and convulsions.

Hepatotoxicity in animals may be the result of DDT and its metabolites' disruption of mitochondrial membranes (Byczkowski, 1977), cell damage, and cell death. Cell regeneration in the liver may lead to hyperplasia, hypertrophy, and the promotion of liver tumors (Fitzhugh and Nelson, 1947; Schulte-Hermann,1974).

The metabolic and reproductive effects of DDT and its metabolites are inter-related. DDT and its breakdown products induce cytochrome P450, which enhances metabolism of endogenous steroids and sex hormones (Nims et al., 1998). Additionally, *p,p'*-DDE can increase levels of aromatase, an enzyme that converts steroids to estrogens, in livers of adult male rats (You et al. 2001). Reproductive effects result when DDT or its metabolites mimic, antagonize, or alter the synthesis or metabolism of endogenous steroid and sex hormones, or alter hormone receptor levels. Several studies suggest that DDT-related compounds may have estrogenic and/or anti-androgenic actions, when present at sufficient concentrations. Guillette et. al., (1994) demonstrated the estrogenic effects of DDT and its metabolites on the reproductive development of American alligators in a pesticide-contaminated lake in Florida. Anti-androgenic effects have also been demonstrated in laboratory rats exposed to *p,p'*-DDE (Kelce et al., 1995).

DDT and related compounds have been shown to cause cancer in some studies of laboratory animals. However, the evidence for DDT causing cancer in humans is inconclusive; several studies have reported a possible causal relationship between DDT exposure and breast cancer (Dees et al., 1996, 1997a, 1997b; Shekhar et al., 1997; Zava et al., 1997). These studies have revealed that DDT compounds increase cell proliferation in human breast cancer cells; this effect can be blocked by anti-estrogenic compounds.

Given the toxic effects of DDT and its persistence in the environment, it has been listed as one of the "Dirty Dozen" POPs in the Stockholm Convention. The Stockholm Convention on Persistent Organic Pollutants was completed on May 23rd, 2001 in Stockholm, Sweden and became international law on May 17th, 2004. The convention allows for restricted use of DDT for mosquitoe control in countries trying to limit impacts due malaria.

Chlordanes

The term "chlordane" refers to a mixture of related compounds, including trans-chlordane, cis-chlordane, , heptachlor, trans-nonachlor and cis-nonachlor. Chlordane mixtures have also been referred to as Octachlor and Velsicol 1068. In the United States, chlordane first as a pesticide in agriculture and for termite control in 1948. The use of chlordane on crops was restricted between 1978 through1983, when it was banned from agricultural use in the United States. However, it continued to be used for residential termite control until 1988 (ATSDR 1994). Although the domestic use of chlordane was banned in 1988, it was produced for international export until 1997.

Similar to DDT and other organochlorine pesticides, chlordane is lipid soluble and highly persistent in the environment. Chlordane mixtures can be detected in treated soils ≥ 10 years post-treatment (Bennett, 1974). Due to its ability to bioaccumulate, its lipophilic nature and its toxic effects, chlordane was included on the Stockholm Convention list of the "Dirty Dozen" persistent organic pollutants. The following is summarized from the U.S. Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease Registry, 1994, Toxicological Profile for Chlordane (http://www.atsdr.cdc.gov/toxprofiles/tp31-p.pdf):

Exposure to chlordane can result in neurotoxicity, immunotoxicity and cancer. Hrdina et al. (1974) observed tremor, paralysis, and tonic-clonic convulsions in rats with a single oral dose of 200-300 mg/kg of chlordane. Inoue et al. (1989) found that chlordane produces neurotoxicity by altering the release of norepinephrine. *In vitro* immunotoxicity studies have suggested that *trans*-chlordane and its metabolites suppress both cell-mediated and humoral immune responses (Johnson et al., 1987). Studies with mice have demonstrated that chlordane can cause liver cancer (Khasawinah and Grutsch, 1989). Early epidemiological studies did not provide conclusive results that chlordane causes cancer in humans (MacMahon et al., 1988; Shindell and Ulrich, 1986), although more recent studies have reported associations between chlordane exposure and non-Hodgkin's lymphoma (Colt et al., 2006).

Recent studies have investigated the toxic effects of chlordane in humans. Colt et al., (2006) found that there was an increased risk (odds ratio, 1.3; 95% CI, 1.0-1.6) of developing non-Hodgkin's lymphoma for people who lived in homes that had been treated for termites before the 1988 ban on chlordane. In 2004, Reed et al. provided evidence of the immunotoxic effects of chlordane-related compounds. Reed et al. investigated the *in vitro* effects of pesticides on human natural killer (NK) cell cytotoxic function and found that alpha-chlordane, gamma-chlordane, *p,p'*-DDT, heptachlor, oxychlordane, and pentachlorophenol (PCP) reduced human NK cytotoxic function after a 24 hour exposure. In 2006, Beach and Whalen reported that induction of interleukin-producing T cells helped counteract oxychlordane and PCP's suppression cytotoxic function in natural killer (NK) cells. In addition, several recent studies have

demonstrated that chlordanes can serve as endocrine disruptors in humans (Lemaire, 2006; Dehn, 2005), male green neon shrimp (Huang, 2004), red-eared slider turtle (Willingham, 2004), and loggerhead sea turtles (Keller, 2006).

Butyltins

Organotins, or butyltins (BTs), are a group of organometallic compounds that were first synthesized in the 1930s, but did not gain wide commercial use until the 1960's and beyond (Tanabe, 1999). These compounds were developed to be used as antifouling paints for a wide range of maritime activities. Chief among these is tributyltin (TBT), which is metabolized to dibutyltin (DBT) and monobutyltin (MBT). The world annual production of organotins has been estimated at 50,000 tons (Fent, 1996). Widespread application and environmental persistence of organotins has prompted a range of studies to examine their potential negative impacts on aquatic systems (Alzieu , 1986; Beaumont and Newman, 1986; Alzieu 1991; Tanabe, 1999). A wide range of impacts have been reported for marine diatoms and invertebrates, including growth reduction of marine microalgae (Beaumont and Newman, 1986), shell thickening and spat failure in oysters (Alzieu et al., 1986; Alzieu, 1991) and imposex in gastropods, such as whelks (Bryan et al., 1986; 1987). In 1988, the federal government enacted the Organotin Antifouling Paint Control Act, which prohibited the use of butyltin paints on boats shorter than 25 feet long, except for aluminum boats. Additional regulations were enacted in 1990 that limited the leaching of butyltins from bottom paint to no more than 4 mg/cm^2/day for boats longer than 25 feet and required that certification be required to perform the application of butyltin paints. Control measures have now been implemented in most industrial countries.

In marine mammals, BTs have been widely detected in cetaceans (Kannan et al., 1996; Kim et al., 1996; Kannan et al., 1997; Madhusree et al., 1997; Tanabe et al., 1998; Tanabe 1999, Kannan et al., 2005; Yang et al., 2006; Yang and Miyazaki, 2006), pinnipeds (Tanabe, 1999; Wolkers et al., 2004) and mustellids (Kannan et al., 1998). The highest concentrations are typically found in liver, kidney, blubber and hair; shedding may provide an efficient route for organotin excretion for pinnipeds (Tanabe, 1999). In contrast, cetaceans appear to have limited routes for organotin excretion (Tanabe, 1999). In a prior study (Tanabe, 1999), male-female differences were less marked for organotins, suggesting that transplacental and lactational transfer were less significant routes of exposure than for most other POPs. Differences in organotin levels between coastal and offshore-dwelling cetaceans have also been noted, with coastal species having substantially higher liver concentrations (Tanabe, 1999). In this same study, hepatic concentrations of TBT and DBT in coastal cetaceans exceeded toxic threshold levels established in laboratory animals (Tanabe, 1999).

A wide variety of toxic effects that have been documented in vertebrates, including hepatotoxicity (Ueno et al., 1994), one especially important finding is a strong association with immunological dysfunction, even at low concentrations (Fent, 1996; Heidrich et al., 2001; Whalen et al., 2002). Potential cellular and subcellular mechanisms of induction of toxicity by organotins include inhibition of CYP 450 (Kim et al., 1998; Heidrich et al., 2001), inhibition of NK cell cytotoxicity (Whalen et al., 2002; 2003), embryotoxicity (Fent, 1992), decreased cyclic AMP in lymphocytes (Whalen et al., 2001 a,b) and decreased phagocyte or hemocyte function in vertebrates and invertebrates (Bouchard et al., 1999; Cima et al., 2002; St-Jean et al., 2002a; 2002b)

PAHs

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous contaminants from both anthropogenic activities and natural sources (e.g., oil seeps, forest fires). They are also the most toxic component of petroleum for both acute and chronic exposure of humans and animals, with single-ring, benzene-like compounds being the most acutely toxic. Engine exhaust, street runoff, sewage and industrial outfalls, oil spills, coal-fired power plants, shipping, legacy pollutants and natural geological deposits all contribute PAH to the marine environment. Anthropogenic sources predominate in populous coastal areas in the absence of major oil seeps. In southern California, the presence of numerous oil seeps in the Santa Barbara Channel and elsewhere are the largest source of PAH to the ocean, and PAH account for about a third of southern California oils by weight (Reed and Kaplan, 1977). In northern California, where there are far fewer natural seeps, anthropogenic sources predominate, but a creditable PAH budget has not been reported for this area.

PAH are persistent, hydrophobic and lipophilic, so they attach to small particles and disperse widely in the atmosphere and the oceans (Neff, 1979). Because of these properties, PAH are bioaccumulated from food, water and sediments, except when they are tightly bound to large organic matrices, such as coal. However, biologically available PAH are not biomagnified like PCBs and DDTs, as they are metabolized by many animals. Some invertebrates (e.g., bivalves) accumulate PAH faster than they are metabolized, thus passing on their burdens to their predators. The metabolites of PAH can be highly toxic, causing genetic damage and leading to tumors and immune system impairment (Reynaud and Deschaux, 2006). The large investment in chemical carcinogenesis research over the last 35 years has been very useful in clarifying potential effects of PAH on marine animals.

Aromatic hydrocarbons consist of one or more fused 6-carbon benzene ring structures, often with carbon side chains of various lengths. Polycyclic aromatic hydrocarbons consist of ≥ 2 aromatic rings. These compounds do not have as many electrons as a straight-chain saturated hydrocarbon of comparable length (i.e., alkanes). However, some of the existing electrons within the aromatic rings are not localized, thus contributing greatly the molecular reactivity of these molecules. In addition to their biological reactivity resulting from electron exchange, the overall shape of a PAH molecule may facilitate binding to cell the receptors for a number of inter- and intra-cellular signaling pathways. The rings in PAH molecules are flat (i.e., planar), which allows some forms to bind with cell receptors. In addition, the existence of bay regions (indentations) in the structure of some multi-ring PAH enhance the reactivity of their metabolites with key cell molecules, such as DNA.

The same cellular receptors that respond to PCBs, the aryl hydrocarbon receptors (Ah), also respond to PAHs, inducing CYP 450 protein induction. It is these proteins that catalyze the addition of oxygen to the aromatic rings, transforming PAHs to epoxides. The epoxides then further rearrange to form dihydrodiols that can undergo further epoxide formation, resulting in a cascade response. Some dihydrodiols of PAHs, such asbenzo(a)pyrene 7,8-dihydrodiol-9,10 epoxide, will bind to DNA, causing mutations and tumors (Klassen et al., 2001).

Sea otters can be exposed to PAHs in several ways. If there is an oil spill or even a substantial accumulation of PAHs in the surface microlayer of the ocean, PAHs will be adsorbed to the sea otter's fur (Guitart et al., 2007; Wurl and Obbard, 2004). As sea otters spend most of their life at the ocean surface, this surface contamination, although seemingly minor, can be a source of chronic exposure for otters foraging just offshore of urbanized areas. These compounds may also be ingested during grooming, inhaled or may pass through intact skin with prolonged contact. In urban areas, combustion sources, specifically automobile exhaust have been shown to be the greatest contributor of PAH to the surface microlayer (Zeng and Vista, 1997; Pereira et al., 1999: Dickhut et al., 2000). In an oil spill setting, surface slicks will facilitate wetting of the pelage, resulting in loss of the protective air layer; hypothermia and a negative energy balance if a significant portion of the coat is affected (Kooyman and Costa, 1978). If the affected area of the hair coat is small, but is in constant contact with cold water, significant negative impacts may still occur. Since most bivalves accumulate bioavailable hydrocarbons, they are an additional source of PAH exposure for sea otters. Finally, otters may encounter PAH deposited in marine sediments from previous oil spills while digging for food in bottom sediments, thereby liberating free oil.

In San Francisco Bay PAH increased sharply during the 20th century commensurate with coastal urbanization (Pereira et al., 1999) and similar trends are likely elsewhere in coastal California. On a shorter time scale (30 years), the National Mussel Watch monitoring program has demonstrated that higher molecular weight PAH originating mainly from combustion have decreased in monitored mussels nationwide between 1988 and 1993 (O'Connor and Lowenstein, 2006). Analyses of individual mussel collection sites within the southern sea otter range in Central California have not demonstrated the same declining trends over the same time period. However, mussels collected from 4 four additional sites between Bodega Bay and Eureka exhibited increases in PAH levels during the same time period. A study of PAH concentrations in sediments from the vicinity of Moss Landing in Monterey Bay, California reported PAH concentrations between 20-120 $\mu g\ kg^{-1}$ for sediments from collected just offshore, 1400-3000 $\mu g\ kg^{-1}$ for those collected within Moss Landing Harbor and 150-375 $\mu g\ kg^{-1}$ for sediments sampled inside of Elkhorn Slough between 1985 and 1987 (Rice et al., 1993).

Since sea otters rapidly metabolize PAHs, tissue concentrations of the parent compounds (unmetabolized) are not a reliable index of exposure; significant chronic exposure to PAH contamination may occur without the accumulation of unmetabolized PAHs in the liver. Although inducible cytochromes such as CYP 450 also respond to contamination by PCBs and DDTs, these contaminant-inducible CYP 450 gene products or their enzymatic activities may be serve as more accurate indicators for PAH exposure than are direct measures of PAH concentrations in tissue.

MATERIALS AND METHODS

Carcass collection, necropsy and tissue archival

Dead-stranded southern sea otters were collected along the central California coast, refrigerated and transported to the California Department of Fish and Game (CDFG) Marine Wildlife Veterinary Care and Research Center (MWVCRC) in Santa Cruz, California for examination. All otters received a complete gross necropsy and microscopic examination of major tissues

including heart, lung, liver, kidney, spleen, stomach, small intestine, colon, omentum, thymus, thyroid, parathyroid, adrenal gland, pancreas, pituitary, multiple lymph nodes, skeletal muscle, reproductive tract, gonads and brain. Tissue sections were placed in 10% neutral buffered formalin, paraffin-embedded, sectioned at 5 µm and stained with hematoxylin and eosin for examination by light microscopy. Acanthocephalan parasites known to infect sea otters (Hennessy and Morejohn, 1977) were identified to genus by overall size, attachment characteristics and proboscis morphology at the time of gross necropsy (Amin, 1992). Evaluation of bacterial, fungal, and parasite samples and testing for the presence of biotoxins were performed when indicated. Swabs for bacterial culture were plated on tryptic soy agar with 5% sheep blood, MacConkey agar, and XLT-4 agar (Hardy Diagnostics, Santa Maria, California) and were incubated at 37 ºC. Bacterial, fungal and protozoal isolates were identified at the UC Davis School of Veterinary Medicine using standard biochemical and molecular techniques.

Causes of death were rigorously standardized so that the primary cause identified for each otter was the most substantial injury or illness leading directly to death. Both the primary and first 2 contributing causes of death were considered in epidemiological assessments: details on scoring criteria and case selection are provided below. Only otters that were in good postmortem condition (postmortem interval <72 hours) were included in this study; otters with incomplete histopathology were excluded. Animals that stranded alive and remained in rehabilitation for ≥ 2 weeks prior to death were also excluded due to concerns regarding changes in POP tissue profiles during captive care and artificial feeding.

During necropsy, the liver was examined and removed, placed on a plastic cutting board and sectioned for sample collection using knives or scalpels made of carbon or stainless steel. All necropsy equipment was cleaned with soap, hot water, and disinfectant between animals and scalpel blades were not re-used. At least two aliquots of liver ≥ 30 g each were collected at necropsy. Samples collected between 2000 and 2001 were wrapped in aluminum foil and stored in WhirlPak bags (M-Tech Diagnostics Limited, Cheshire, England), or were placed directly into the bags. After 2001, samples were wrapped in Teflon sheets prior to placement in WhirlPak bags. Archived samples were labeled with the necropsy date, sea otter number, necropsy number, tissue type, packaging type, and sample ID number and were frozen at -80ºC until analyzed.

Each otter's total length was measured to the nearest 0.5 cm and body weight was determined to the nearest 0.01 kg. Nutritional condition was defined both as a subjective assessment of the volume of subcutaneous body fat (scored as none, scant, fair, moderate and abundant) as well as by assessing overall nutritional condition, including amounts of muscle and body fat, relative prominence of the ribs and the presence or absence of inanition-related atrophy of internal organs (scored as emaciated, poor, fair, good, or excellent). The occurrence of food in the gastrointestinal tract and the ingested prey species were also recorded by biologists or pathologists.

Age was determined using previously established criteria, based on dentition, total length, pelage characteristics, presence or absence of open skull sutures and relative prominence of the sagittal and lambdoidal crests. Each otter was grouped into one of four biologically-relevant age classes using previously published criteria (Miller et al., 2002b; Kreuder et. al. 2003): immature (6 months to 1 year); subadult (1–3 years old), prime-aged adult (4-9 years) or aged adults (≥ 10

years). Although analysis of cementum annuli in premolar teeth is performed in southern sea otters, this ageing technique has yet to be validated using known-aged otters. Sea otter pups were excluded from this pilot study due to cost constraints and a desire to focus available funds and diagnostic effort on the most critical component of the threatened southern sea otter population (animals within or approaching their maximal reproductive years). In addition, overall POP concentrations in sea otter pups represent maternal transfer of as a result of mobilization of tissue POP stores during pregnancy and lactation, and not pollutant burdens acquired from active foraging. However, we hope to include this last segment of the sea otter population in subsequent POP studies.

Archived liver samples from all available freshly dead otters (immatures, subadults, adults and aged adults) necropsied between January, 2000 and April, 2005 were included in the study without selection bias with respect to each otter's stranding location, sex or necropsy findings. All samples selected for POP testing were placed on dry ice and hand-carried to the CDFG Water Pollution Control Laboratory in Rancho Cordova, California for POP testing.

Sample population: demographics, distribution and findings from necropsy and histopathology

Sea otters eligible for enrollment in the study included all freshly dead southern sea otters (excluding pups) stranding between January, 2000 and April, 2005 that were submitted to CDFG-MWVCRC for necropsy. Each sea otter's stranding date was used for time-based comparisons.

After microscopic examination of all tissues, the three most significant findings were ranked by overall importance as an immediate cause of each animal's death by a pathologist with no knowledge of the individual POP test results. Each sea otter was then assigned to one of 7 major mortality categories:

- Trauma without concurrent infectious disease
- Trauma with concurrent infectious disease
- Otters with protozoal infection as a major finding, but without trauma
- Otters with acanthocephalan peritonitis as a major finding, but without trauma
- Otters with bacterial infection as a major finding, but without trauma
- Otters with other infectious or potentially infectious processes other than the 3 above, such as coccidiomycosis or idiopathic brain inflammation, but without trauma
- Otters with POP test results that did not fit any of the above 6 criteria (miscellaneous cases)

To facilitate studies regarding associations between specific infectious diseases and liver POP levels (such as acanthocephalan peritonitis, protozoal meningoencephalitis or fungal or bacterial disease), additional data columns were added for each major category of infectious disease and each otter was coded as "absent" or "present" for that disease category, regardless of concurrent disease processes. Trauma was also included as a separate column to facilitate investigations of whether trauma and concurrent infectious disease acted as covariates with respect to tissue POP concentrations.

Associations of POP test results with spatial and environmental risk factors
Several environmental variables were derived from sea otter stranding locations to help determine the association between urban development and types of discharges on sea otter POP concentrations and causes of death. To assign a numerical value for each otter's stranding or sampling location, the central California coastline encompassing the southern sea otter range (661 km) was divided into 0.5 km increments and was assigned a numerical value, starting at 1 to the north and ending at 1322 to the south (CDFG, Unpub. data). Each point was mapped in reference to prominent coastal geographical features along a hand-smoothed line, set offshore at five fathoms water depth. All live or dead otters sampled along the coastline were assigned to the closest 0.5 km site, based on their location at the time of carcass recovery or capture. These data were then categorized into 5 coastal segments: north coast (1-246), Monterey Bay (247-390), Big Sur coast (391 – 787), Estero Bay (788 – 852) and south coast (853-1322) (Figure 1).

Assigned values for human coastal human population density were derived from United States 2000 census data (United States Census 2000, www.geographynetwork.com), and reflect the relative human population density at each coastal stranding location (e.g., 1 = 0 to 100, 2 = 101 to 1000, 3 = 1001-3000, 4 = 3001-6000 or 5 = >6000 humans per square mile), using the human population density score of the adjacent census tract (Figure 1). Census Bureau tracts are relatively permanent geographic subdivisions within a county or equivalent entity.

Comparison of the effects of freshwater outflows and wastewater discharges on liver POP burdens was accomplished using previously described estimates (Miller et al., 2002b). The freshwater data use 60-year average rainfall (Teale Data Center, 1997), expressed as areas of equal rainfall, or isohyets, encompassing the total surface area of each watershed, to estimate the total discharge from each watershed. Coastal freshwater outflow (via streams and rivers), was expressed as total annual outflow (acre-feet/year), at each site, assuming that all rainfall flowed to the ocean. Four categories were used categorize the relative exposure to freshwater outflows to the nearshore marine environment, based on each sea otter's stranding location: 0 to 10,000, 10,001 to 100,000, 100,001 to 1,000,000, or greater than 1,000,000 acre-feet/year (Figure 2). However, no sea otters in the present study stranded at points with freshwater outflows greater than 1,000,000 acre-feet per year. The proximity of each otter's stranding site to the location of the nearest major municipal wastewater outfall was determined as described (Miller et al., 2002b) using the same technique as for freshwater discharges. Wastewater plant discharge locations and volumes were obtained from the National Pollutant Discharge System (NPDES) permit records from the California Central Coast Regional Water Quality Control Board. For each plant, total yearly marine discharge (in acre-feet per year) was used. Wastewater outflow was categorized as <1, 1 to 4,000, 4,000 to 8,000 and >8,000 acre-feet/ year (Figure 3). However, no sea otters in the present study stranded at points with wastewater outflow >8,000 acre-feet/ year. Each otter's exposure to freshwater and wastewater discharges was estimated by comparing the stranding location to the estimated concentrations of freshwater and effluent at that location, based upon a simple exponential dilution from the point of discharge;no attempt was made to correct for seasonal variations in the volume of water discharged at each site or local effects attributable to wind, marine currents or coastal geography.

Figure 1. Coastal human population density (persons/ mile^2) by coastal census tract (Source: United States census, 2000).

Figure 2. Maximum freshwater runoff from major coastal streams and rivers, central California (acre-feet/year).

Figure 3. Maximum coastal municipal wastewater outflows, central California (acre-feet/year).

Analysis of liver concentrations of POPs

Sea otter livers were tested for 138 POPs, including polycyclic aromatic hydrocarbons, organotin compounds, and synthetic organic compounds, such as chlorinated pesticides, polychlorinated biphenyls, and polybrominated diphenyl ethers. A complete list of the compounds tested is presented in Appendix A. Analyses were performed at the California Department of Fish and Game Fish And Wildlife Water Pollution Control Laboratory using the following procedures.

Polycyclic aromatic hydrocarbon compounds.

Ten-gram aliquots of homogenized tissue were fortified with surrogate compounds and extracted using EPA Method 3545 Pressurized Fluid Extraction (Dionex ASE 200) with acetone: dichloromethane (1:1) using heat and pressure. Sample extracts were cleaned up using EPA 3640A Gel Permeation Chromatography (GPC) followed by Alumina/Silica column chromatography cleanup using EPA 3610 B - EPA 3630 C modified. The extracts were evaporated to a final volume of 0.5 mL in isooctane using nitrogen. Sample extracts were fortified with internal standards and analyzed for PAH compounds by GC/MS SIM using an Agilent 6890 gas chromatograph equipped with a 5973 *Network* "Inert Source" Quadrapole Mass Selective Detector and a 60 meter DB5-MS (J&W Scientific) (0.25 mm I.D. and 25 μm film thickness) fused silica column.

Organotin compounds

Ten-gram aliquots of homogenized tissue were fortified with TBT-d_{27} (surrogate) and mixed with a derivitizing agent (sodium tetraethylborate [$NaBEt_4$]), a buffer (acidified sodium acetate) and solvent (methanol). Hexane was then added and the samples were allowed to react overnight. The mixture was centrifuged and the organic layer was removed from the centrifuge tube, dried with sodium sulfate and cleaned-up using a 2 gram silica gel column with hexane.The collected extract was concentrated by roto-vap and the volume adjusted to 2 ml with hexane.An organotin standard was also derivitized with each set of samples. The final extracts were analyzed using an Agilent 6890 gas chromatograph configured with a single injector and two 30 meter capillary columns of differing polarities (DB5 and DB17) and dual flame photometric detectors equipped with tin filters (tin mode).

Synthetic organic compounds in tissue

Ten-gram aliquots of homogenized tissue were prepared as described for PAHs above, with the exception of fractionation using EPA 3620B (modified) florisil column chromatography. The extracts were fractionated into three parts using 6% diethyl ether/petroleum ether, 15% diethyl ether/petroleum ether and 50% diethyl ether/petroleum ether and each fraction was evaporated to a final volume of 1.0 mL. Sample extracts were fortified with internal standards and analyzed for PCB congeners by EI-MS-MS using a Varian 1200 triple quadrupole mass spectrometer. The organochlorine pesticides were analyzed using two Agilent 6890 gas chromatographs configured with a single injector, two 60-meter capillary columns of differing polarities (DB5 and DB17) and dual micro-electron capture detectors.

Data analysis for liver POPs and sea otter stranding and necropsy data

All animal information (stranding location, demographics and necropsy results) and contaminant levels were transcribed onto multiple spreadsheets to facilitate analyses (Excel®, Microsoft, Redmond, WA USA). Using these contaminant levels, toxic equivalencies (or TEQs) for PCBs

were calculated for each sample as previously discussed (Van den Berg et al., 1998; Safe, 1990). Briefly, this method quantifies the bioactive PCB contaminants in the liver tissue by comparing the result to 2,3,7,8-TCDD as follows:

$$TEQ = \Sigma\{([PCB_1] \times TEF_1) + ([PCB_2] \times TEF_2) + \ldots + ([PCB_x] \times TEF_x)\}$$

where $[PCB_x]$ is the tissue concentration of PCB_x and TEF_x is the toxic equivalency factor for that specific PCB, arrived at by international consensus evaluating a number of species and toxic endpoints (Ahlborg et al., 1994). Additionally, as a second estimation to attempt to capture the additive effects of multiple contaminants, the sum of the percentages of available mammalian LD50 concentrations for assessed contaminants was calculated in a similar manner to TEQ.

$$\%LD50 = \Sigma\{([POP_1]/LD50_1) + ([POP_2]/LD50_2) + \ldots + ([POP_x]/LD50_x)\}$$

where $[POP_x]$ is the tissue concentration of POP_x, and $LD50_x$ is the lethal dose for 50% of animals exposed to POP_x in mink (or in rats if mink data for mink was not available) (Parametrix, 2001; UNEP, 2006; World Health Organization, 2006).

Data (and standard transformations) were assessed for normality of distribution by evaluating skewness, kurtosis and Shapiro-Wilk test statistics. Based on these results, all data were transformed using a LN (X+1) transformation as previously reported (Sokal and Rohlf, 1995) and parametric statistical methods were used. Basic descriptive statistics (means, medians and standard deviations) for all data, as well as subcategories stratified by age class, location, subcutaneous (SQ) fat stores, sex and major findings at necropsy were calculated using standard methods. Differences between demographic (age, sex, location and SQ fat) categories were evaluated using ANOVA methods and, for those significant differences, subsequent pairwise comparisons (Tukey) were done. Generalized linear modeling (GLM), with the contaminant/sum contaminant level as the dependent variable and significant demographic categories as independent variable(s) were used to generate multivariate models to best evaluate impacts of these factors on contaminant burdens.

The impact of contaminant burdens and demographic categories on the presence of disease in necropsied otters was evaluated using Student's t-tests and chi-squared analyses, respectively, followed by logistic regression (LR) methods for those factors deemed statistically significant. Crude odds ratios (and associated 95% confidence intervals) were determined using univariate LR, with disease as the dependent and the individual contaminant as the independent variable. Adjusted odds were then determined for contaminants by forcing all demographic parameters (age, sex, location and nutritional condition, as determined by SQ fat stores) into the model. In this manner, all contaminants that were indicated as significant risk factors for various causes of death were adjusted to account for the contributions of demographic factors, such as age class, gender and nutritional condition on liver contaminant concentrations. Lastly, the most parsimonious multivariate logistic model for each disease state was determined through manual stepwise entry of contaminant and demographic parameters into the model and comparison of subsequent goodness-of-fit values between each model and the actual data (calculated as 2 x [log likelihood (new model) – log likelihood (old model)] and comparison to a chi-squared distribution.

For both the linear and logistic regression models, final models (and odds ratios in these multivariate models) should be interpreted in comparison to those animals without that factor.

For example, a finding of increased risk due to no fat is in comparison to all other animals with scant or moderate fat levels.

Mussel Data Acquisition and Manipulation

National Status and Trends (NST) Mussel Watch Project data were acquired from the Center for Coastal Monitoring and Assessment via the internet (http://ccma.nos.noaa.gov/). California State Mussel Watch data were considered, but were not used because matching POP data were not available for the time interval of 1995 through 2004, encompassing the period of ≥5 years prior to sea otter tissue sampling for the same areas. All available NST data for the following California locations were downloaded: Point Santa Cruz, Moss Landing, Elkhorn Slough, Lover's Point (Pacific Grove), San Simeon Point, Point San Luis and Point Conception. These data were imported from a text file into Excel and then into Microsoft Access (Microsoft Corp, Redmond, WA USA). In Access, the dry weight mussel contaminant data were converted to wet weights using the following formula: dry weight X (100 - % moisture / 100) = wet weight. The percent moisture data were also obtained from from the NST website. After converting the dry weight (DW) data to weight wets (WW), the Moss Landing and Elkhorn Slough data were averaged to facilitate comparisons with POP data from sea otters stranding in in this general area. Wet weight POP data for mussels were compiled as individual analytes and were pooled into major contaminant groups, including sumPCBs, sumChlordanes, sumHCHs, and sumDDTs. These pooled and non-pooled WW mussel data were then averaged for each five year period preceeding the year of stranding for each sea otter. For example, contaminant data for mussels sampled from Point Santa Cruz between 1995 and 1999 were averaged for an otter that stranded near Point Santa Cruz in 2000. Most Mussel Watch sites were sampled every other year, therefore average values over a five year time frame were often derived from two or three sample periods. Excel spreadsheet formulas were used to calculate the closest mussel site to each sea otter's stranding location; preliminary analyses demonstrated that using this approach yielded the best model, when compared to using an exponentially weighted average of all mussel site data (data not shown). The five-year average of POP concentrations for the closest mussel site to each applicable sea otter stranding location were used as the independent, continuous covariate for analyses of covariance (ANCOVA), with sea otter liver POP concentrations as the dependent variable. As histograms, boxplots, and probability plots demonstrated that most mussel and the sea otter liver POP concentrations (wet weight) were not normally distributed, the LN (x+1) transformation normalized the sea otter and mussel POP data relative to the sumDDT, sumChlordane, sumHCH, HCB, dieldrin, and sumPCB values.

Mussel Data analyses

An analysis of covariance on sea otter POP concentrations was performed using the General Linear Model. Of 227 wild sea otters tested for POPs, three animals from north of Half Moon Bay and two from south of Point Conception were excluded from the ANCOVA analysis due to their extended distance from the closest mussel sample sites used for the analysis. Sea otter age class, sex, and nutritional condition were treated as independent, fixed categorical variables. Three-way interactions were not tested due to a lack of immature otters with moderate or abundant body fat. Moderate and abundant body fat categories were combined for the ANCOVA analyses to facilitate tests for two-way interactions between age, sex, and nutritional condition. Mussel POP concentration was treated an independent continuous covariate. Three-way interactions with age, sex, nutritional condition and mussel contaminant concentration were

tested. A separate ANCOVA was conducted for each of the following sea otter POPs: sumDDT, sumChlordane, sumHCH, dieldrin, HCB, and sumPCB.

Statistical Interpretation and Software
All comparisons were deemed statistically significant at a $P < 0.05$. All statistical analyses were done using appropriate software packages (SPSS 10 and 11.0, SPSS Inc., Chicago, IL USA; SYSTAT 11.00.01, Systat Software Inc, San Jose, CA USA).

RESULTS

Sample population: demographics, distribution and exposure to environmental risk factors
Based on our established selection criteria, 227 freshly dead otters were enrolled in the study; three were frozen-thawed, freshly dead animals, and the remainder were non-frozen otters. In addition, liver from three long-term captive otters were tested for POP burdens to compare with the wild animals, but test results from these captive animals were excluded from all statistical analyses.

The 227 freshly dead, wild, stranded otters were composed of 138 males and 89 females, divided into the following age classes: immatures (34), subadults (44), adults (111) and aged adults (38). Age class and sex of stranded sea otters by region are presented in Table 1.

Table 1. Age class, sex and location of sea otters tested for POPs.

Gender / Region	Age class				
Male	Immature	Subadult	Adult	Aged Adult	All Ages
North Coast	0	2	3	1	6
Monterey Bay	8	12	32	13	65
Big Sur	3	0	1	1	5
Estero Bay	6	9	12	2	29
South Coast	3	6	16	8	33
All Regions	20	29	64	25	138
Female					
North Coast	1	2	3	0	6
Monterey Bay	6	3	19	9	37
Big Sur	4	2	10	3	19
Estero Bay	3	6	10	1	20
South Coast	0	2	5	0	7
All Regions	14	15	47	13	89
Grand Total	34	44	111	38	227

The nutritional condition of all animals was considered in our analyses independent of the cause of death. Nutritional condition, assessed as the level of SQ fat, was assessed at gross necropsy, and ranged from none (77 otters), scant (56), fair (17), moderate (34) to abundant subcutaneous

fat (43). The majority of otters (131 of 227) had empty gastrointestinal tracts at the time of necropsy, while 89 had some food in their gastrointestinal tracts. The presence or absence of gastrointestinal tract contents were not noted for seven otters. For otters with food in their gastrointestinal (GI) tract, the most common finding was various species of crustaceans (n=56 otters), followed by marine and estuarine bivalves (n= 23), echinoderms (12), cephalopods (8) and gastropods (2). The vast majority of 227 examined otters had little (39) to no (131) food in their GI tract at the time of necropsy, compared to 49 otters with medium (26) to full (23) GI tracts. For seven additional otters, the volume of food present at necropsy was not recorded. The distribution of otters by sample year was 21 for 2000, 25 for 2001, 31 for 2002, 68 for 2003, 65 for 2004 and 17 for the first quarter of 2005. Sea otters enrolled in the present study were recovered over 946 km of the California coast, ranging from Tomales Point in Sonoma County, to Huntington Beach in Orange County (Figures 4 and 5). Sampled otters were almost evenly split between the northern and southern halves of their range, with 126 otters recovered north of Yankee Point, located on the Big Sur coast, compared to 101 otters recovered from areas southward of this region. When subdivided by major coastal region, 12 sea otters were recovered from the north coastal range, 102 from Monterey Bay, 24 from the Big Sur coast (including the Carmel River watershed and Point Lobos), 49 from Estero Bay (including Morro Bay) and 40 from the coast south of Morro Bay. When the distribution of sea otters was examined in relation to proximity to freshwater runoff, about half (55.5%, n=126) were recovered from areas of low freshwater outflow, compared to 75 otters (33%) exposed to moderate outflow and 26 (11.5%) that were exposed to heavy freshwater outflows. Similarly, when the sea otter stranding distributions were examined relative to proximity to major municipal wastewater outfalls, nearly 75% of the otters (164) were obtained from areas with low exposure to municipal wastewater, while the remainder were exposed to moderate levels of municipal wastewater. No otters in the present study were recovered from areas known to be heavily exposed to municipal wastewater. Approximately 19% of the otters (43/227) were from coastal areas exposed to both moderate to high freshwater runoff and moderate municipal wastewater. With regard to coastal human population density, most of otters examined (176/227) were recovered from areas of low to moderate coastal human population density. Specifically, just under half of the otters (106) were recovered from areas with $\leq$ 1,000 persons/ mile^2; compared to 70 otters recovered from areas with 1,001 and 3,000 persons/ mile^2; 23 otters recovered from areas with 3,001 to 6,000 persons/mile^2 and 28 otters recovered from areas with >6,000 persons/ mile^2. Gender distributions were roughly equal between the different categories of coastal human density, except at densities of 3,000 to 6,000 persons/ mile^2, where females were slightly under-represented (data not shown).

Figure 4. Stranding sites of fresh dead female sea otters necropsied between January, 2000 and April, 2005.

Figure 5. Stranding locations for fresh dead male sea otters necropsied between January, 2000 and April, 2005.

Findings from necropsy and histopathology
Over 31% (71/ 227) of otters died with meningoencephalitis as a primary or contributing cause of death; of these, 23 had idiopathic meningoencephalitis, 30 had *Sarcosystis. neurona*-associated disease, 13 had *T. gondii*-associated disease and 5 had pathology attributed to both *S. neurona* and *T. gondii*. For 37 otters, protozoal disease was felt to be the most significant finding at necropsy, compared to 20 otters where protozoal disease was a secondary finding, and 14 where it was ranked as a tertiary finding.

Significant, bacterial-related disease processes were detected in ≥ 52% of the otters enrolled in this study (n=119). Bacterial disease was associated with a wide range of lesions, including intestinal volvulus, cardiac disease, dental disease, acanthocephalan peritonitis and pneumonia. For the majority (69%) of cases, bacterial disease was considered as a secondary (69) or tertiary (21) cause of death. However, 29 otters had primary bacterial infections.

Just under 25% of the otters (n=55) had significant acanthocephalan peritonitis. For most of these otters (n = 36), acanthocephalan infection of the abdomen was considered the primary cause of death, either as a direct result of the parasites or due to secondary factors, such as bacterial infection or intestinal perforation. Of 227 otters enrolled in this study, almost 80% (n= 178) had various infectious agents, including bacteria, parasites or fungi listed as a primary, secondary or tertiary cause of death.

Trauma of any type was identified as a significant finding in 94 otters. Causes of trauma in sampled sea otters include shark bite, boat strike, gunshot, intraspecific aggression and entanglement in fishing gear. Trauma was established as the primary cause of death for 76 otters, compared to 19 otters with trauma as a contributing cause of death. However, prior studies have suggested close associations between death due to trauma in sea otters and pre-existing infectious disease, such as protozoal meningoencephalitis (Kreuder et al., 2003). Thus the placement of trauma as the primary cause of death is debatable when moderate to severe, pre-existing infectious disease, especially brain disease, is detected in the same animals. For this reason, we distinguished between otters with trauma and no concurrent disease and otters with both conditions prior to data analysis.

Of 227 sampled otters, 32 had severe trauma without evidence of concurrent infectious disease and 57 had concurrent trauma and infectious disease. In contrast, 29 otters had significant protozoal disease without trauma, 36 had acanthocephalan peritonitis without trauma, 32 had bacterial disease with no trauma, 3 otters had other processes attributed to unidentified infectious agents and 38 died from other, non-infectious and non-traumatic processes, including gastrointestinal impactions and domoic acid intoxication. Cause of death categories are summarized by sex and stranding location in Table 2. For analyses of associations between liver POP levels and the major categorical causes of sea otter death, the following categories were used:
1. Trauma without concurrent infectious disease
2. Otters with protozoal infection as a major finding, regardless of trauma status
3. Otters with acanthocephalan peritonitis as a major finding, regardless of trauma status
4. Otters with bacterial infection as a major finding, regardless of trauma status

5. Otters with any of the above infectious diseases as a major finding (combined category for groups 2-4 above)
6. Otters not included in any of the above categories (miscellaneous cases).

Table 2. Specific infectious diseases and trauma as major findings (primary or major contributing cause of death) in southern sea otters, stratified by location and gender. >1 infectious disease and/ or trauma were sometimes present concurrently.

Gender Region	Number of Sea Otters with Each Diagnosis			
Male	Meningoencephalitis or Systemic Protozoal Infection	Bacterial Infection	Acanthocephalan Peritonitis	Trauma
North Coast	1	3	0	5
Monterey Bay	21	36	21	22
Big Sur	0	2	1	1
Estero Bay	12	14	10	9
South Coast	15	17	6	14
Female				
North Coast	3	2	1	4
Monterey Bay	9	25	9	13
Big Sur	2	10	2	13
Estero Bay	7	7	6	10
South Coast	1	3	0	4
Grand Total	71	119	56	95

POP test results for southern sea otters

Numerous contaminants were detected in the sea otter liver samples. Of the 138 compounds tested, 117 were detected in otters. As a group, DDTs had the highest mean concentrations, followed by PCBs and PBDEs (Table 3). The POP analyte detected at the highest concentration was p,p' DDE, with a mean 614 ng/g wet weight. The next highest compound was PCB 153, with a mean of 30.3 ng/g wet weight. PAHs were found in very low concentrations, with only 33 of 47 PAHs being detected and only 11 had average concentrations greater than 0.1 ng/g.

Table 3. Concentrations of major groups of contaminants in sea otter livers.

	Mean	Minimum	Maximum
Sum of Butyltins	37.67	0	1546.5
Sum of Chlordanes	20.90	0	329.323
Sum of DDTs	628.24	4.85	7636.446
Sum of HCHs	9.54	0	186
Sum of PAHs	3.28	0	55.7
Sum of PBDEs	47.70	0	834.724
Sum of PCBs	175.67	1.573	3940.829

Associations of POP test results with otter location, age, sex and nutritional condition

Sex

Univariate analyses

Based on single factor ANOVAs, significant associations were detected between the concentrations of several POPs in otter livers and otter gender. Of 22 POPs (excluding sumPBDEs) that were significantly affected by otter sex, roughly half (n = 10) of were present in lower concentrations in livers of female otters, when compared to males (Table 4). DDT metabolites were 27-56% less concentrated in females, and certain PBDEs (including summed PBDEs and PBDEs 047 and 100) were 40 to 43% less concentrated in females. Tetrachlorinated PCB congeners were also 33 to 67% lower in female otters. In contrast, more heavily chlorinated PCB congeners were 45 to 287% more concentrated in livers of female otters, when compared to males (Table 4).

Multivariate analyses

A separate multifactor ANOVA was conducted using sumPBDE as the dependent variable and sex, age, body condition, and regional location as fixed categorical variables. The results of the multifactor ANOVA demonstrated significant interactions between sex and age and between sex and region, in addition to the significant effect of sex as a single factor ($p<0.05$). Please see section on POP testing in mussels below for additional ANCOVA analyses on POP associations by sea otter gender.

On multivariate regression analyses, sea otter sex was associated with the hepatic concentrations of the following POPs: p,p' DDD, p,p' DDE, biphenyl, sumPBDE, and sumPCB008-101 (Table 5). For these POPs, males had higher liver concentrations than females, with the exception of biphenyls, which were lower in males than in females. Multiple regression analyses were not conducted for individual PCB or PBDE congeners.

Age Class

Overview

Liver POP concentrations were found to vary significantly by sea otter age class (Table 6). Age class-specific patterns for POP concentration varied between major POP groups, including pesticides, organotins, PAHs, PBDEs, and PCBs. Two distinct patterns of POP concentration by otter age class were detected: Aged adults had significantly higher liver concentrations of organotins and acenaphthylene, compared to immature otters. Conversely, immature otters had significantly greater concentrations of several pesticides and PBDE and PCB congeners in their livers than adults.

Table 4. Differences between males and females for liver POP concentrations in southern sea otters, determined using two-tailed t-tests.

	Female		Male				
Sex	Avg	SD	Avg	SD			
N	89		138		P-value	Ratio female:male	Ratio male:female
DDD(o,p')	1.550	3.245	3.562	8.077	0.0055	0.44	2.297522798
DDD(p,p')	6.538	11.782	11.321	20.152	0.0003	0.58	1.731529434
DDE(o,p')	1.431	7.182	2.045	4.586	0.0276	0.70	1.428462827
DDE(p,p')	508.637	989.065	693.238	963.119	0.0005	0.73	1.362933067
HCH, beta	11.330	18.703	8.533	18.339	0.0228	1.33	0.753145654
Heptachlor epoxide	1.821	2.492	1.323	1.849	0.0221	1.38	0.72639546
Oxychlordane	4.367	5.821	3.104	5.884	0.0158	1.41	0.710730914
Monobutyltin	1.893	7.340	0.150	1.736	0.0032	12.62	0.079226937
Biphenyl	0.359	0.926	0.089	0.449	0.0027	4.03	0.248015334
Methyldibenzothiophene, 4-	0.204	0.610	0.059	0.357	0.0151	3.46	0.288736646
PBDE 047	15.773	27.863	27.845	48.395	0.0007	0.57	1.76535562
PBDE 100	4.768	9.493	8.585	14.766	0.0026	0.56	1.80070744
SUMPBDE	34.282	57.518	57.086	105.761	0.0168	0.60	1.6651856
PCB 060	0.043	0.102	0.120	0.185	0.0002	0.36	2.797956872
PCB 066	0.635	0.689	1.275	1.919	0.0000	0.50	2.007626638
PCB 070	0.158	0.238	0.235	0.252	0.0076	0.67	1.489036712
PCB 074	0.274	0.430	0.686	1.310	0.0001	0.40	2.505357531
PCB 153	41.682	111.434	23.348	56.125	0.0451	1.79	0.560132676
PCB 194	2.941	5.254	1.962	3.368	0.0265	1.50	0.667217653
PCB 195	1.111	4.328	0.387	0.876	0.0065	2.87	0.348693752
PCB 201	4.254	6.879	2.933	4.623	0.0260	1.45	0.689554979
PCB 203	2.949	4.843	1.840	3.209	0.0040	1.60	0.623926592
PCB 206	1.689	3.533	0.932	1.944	0.0078	1.81	0.55186149

Table 5. Results of multiple regression analyses for each POP contaminant.

Contaminant	Sq. Mult. R	Equation
Cis-chlordane	0.103	.158 - .211(Estero) + .23(Immature) + .17(No Fat)
Trans-chlordane	0.023	.023 + .048(Immature)
DDD, op	0.1944	.769 -.699(Big Sur) -.865(Estero) + .472(Immature) + .417(ShedInFlu Med)
DDD,pp	0.387	1.266 - 1.199(Big Sur) - 1.381(Estero Bay) + .696(Immature) + .377(Subadult) -.581(ModFat)+ .401(Male) + .365(ShedInFlu Med)
DDE, op	0.175	.143 + .462(S. Coast) + .819(Immature) + .349(Subadult) + .274(ShedinFlu Med)
DDE,pp	0.438	3.513 - 1.02(Big Sur) - .642(Estero Bay) + .864(Immature) + .464(Subadult) + 1.665(No Fat) + .796(Scant Fat) + .821(Male)
Dieldrin	0.437	1.377 + .457(Monterey Bay) - .316(Estero Bay) + 1.270(Immature) + .695(Subadult) - 0.255 (Scant Fat) - .676(Mod Fat) + .483(Popn 3001-6000)
HCH, beta	0.539	1.733 + .453 (N. Coast) - .231(Estero Bay) + 1.217(Immature) + .279(Subadult) + .342(No Fat) -.787(Mod Fat)
Heptachlor Epoxide	0.553	.295 + 1.114(Immature) + .669(Subadult) +.207(Adult) + .245(No Fat) - .331(Mod Fat) + .276(Popn 3001-6000)
Hexachlorobenzene	0.102	.435 + .397(Immature)
Mirex	0.133	.026 + .223(Aged) + .183(No Fat)
Cis-nonachlor	0.463	0.16 + .669(N Coast) + .702(Monterey) + .483(S. Coast) + .805(Immature) + .398(Subadult) + .515(No fat) - .631(Mod Fat) + .210(ShedInFlu Med) + .307(Popn 3001-6000)
Trans-nonachlor	0.517	1.04 + .951(N. Coast) + .783(Monterey) + .490(S. Coast) + 1.044(Immature) + .565(Subadult) + .705(No Fat) -.758(Mod Fat) + .252(ShedInFlu Med) + .345(Popn 3001-6000)
Oxychlordane	0.549	.522 + .564(N. Coast) + .511(Monterey) + 1.042(Immature) + .697(Subadult) + .431(No Fat) -.505(Mod Fat) + .305(Popn 3001-6000)
Dibutyltin	0.077	1.092 - .976(Immature) - .629(Subadult) + .43(ShedInFlu Low) + .749(Popn 3001-6000)
Monobutyltin	0.066	.296 + .158(Monterey) - .217(Male) + .176(ShedInFlu Low)
Tributyltin	0.07	1.880 - .871(Immature) - .801(Subadult) - .622(Popn 101-1000)
Sum OT	0.061	2.134 - 1.035(Immature) - .795(Subadult) - .649(Popn 101-1000)
Acenaphthylene	0.144	.121 - .194(Monterey) + .220(S. Coast) + .254(Adult) + .441(Aged) + .198(No Fat)
Biphenyl	0.057	.363 - .145(male) - .101(Popn 101-1000)
Chrysene	0.043	.095 + .201(Mod Fat)
Sum PBDE	0.44	2.069 - .624(Big Sur) + .674(S. Coast) + .775(Immature) + .317(Subadult) + .718(No Fat) - .906(Mod Fat) + .602(Male) - .282(Popn 1001-3000)
Sum PCB 008-101	0.364	1.539 - 1.063(Big Sur) - .577(Estero) + .750(Immature) + .356(Subadult) + 1.266(No Fat) + .62(Scant Fat) + .313(Male) + .418(Popn 3001-6000)
Sum PCB 105-209	0.393	3.375 + .948(N. Coast) + .875(Monterey) + .723(S. Coast) -.824(Adult) - .914(Aged) + 1.481(No Fat) + .592(Scant Fat) + .490(Popn 3001-6000)
Total PCB	0.406	4.314 - 1.116(Big Sur) - .661(Estero) - .717(Adult) - .776(Aged) + 1.549(No Fat) + .638(Scant Fat) - .743(POTWInFlu Med) + .548(Popn 3001-6000)

Table 6. POPs for which sea otter age was associated with POP concentrations in sea otter livers. Reported P-values is overall ANOVA result; ages with the same letter in the Sig? column were not significantly different in subsequent pairwise analyses.

Age	Immature			Subadult			Adult			Aged Adult			
	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	
N	34			44			111			38			P-value
Cis-chlordane	1.221	3.129	A	0.459	1.174	AB	0.277	0.742	B	0.191	0.424	B	0.007
Trans-chlordane	0.088	0.203	A	0.043	0.134	AB	0.016	0.085	B	0.042	0.128	B	0.034
DDD(p,p')	17.584	27.039	A	12.476	23.680	AB	6.035	9.026	B	8.619	14.628	AB	0.022
DDE(o,p')	5.656	12.402	A	2.415	5.356	AB	0.822	1.892	B	0.519	1.129	B	0.000
DDE(p,p')	1226.624	1446.825	A	730.345	1076.279	AB	376.384	539.458	C	666.221	1095.369	B	0.000
Dieldrin	18.989	19.266	A	8.758	11.075	B	4.233	6.107	C	3.765	4.083	C	0.000
HCH, beta	31.247	38.525	A	7.470	6.810	B	5.092	7.419	C	6.043	6.115	BC	0.000
Heptachlor epoxide	4.478	3.423	A	2.016	1.620	B	0.733	0.925	C	0.588	0.818	C	0.000
Hexachlorobenzene	1.543	1.294	A	0.681	0.605	B	0.612	0.734	B	1.092	2.364	B	0.000
Mirex	0.426	1.011	AB	0.000	0.000	C	0.128	0.444	B	0.572	0.971	A	0.000
Cis-nonachlor	8.065	13.608	A	3.812	6.791	B	1.527	2.885	C	2.362	3.481	BC	0.000
Trans-nonachlor	30.979	43.327	A	14.728	20.374	B	6.646	10.097	C	9.959	14.532	BC	0.000
Oxychlordane	8.919	8.393	A	5.288	8.113	B	1.752	2.550	C	2.277	3.320	C	0.000
Dibutyltin	3.124	11.481	B	5.143	9.140	AB	23.134	89.828	A	25.825	80.135	A	0.002
Tributyltin	5.309	12.514	C	7.427	13.572	BC	24.661	73.246	AB	31.594	83.068	A	0.001
SumOT	8.432	23.442	B	12.570	21.645	AB	48.807	165.011	A	59.571	170.640	A	0.003
Acenaphthylene	0.225	0.561	B	0.367	0.918	B	0.823	1.495	AB	1.178	1.425	A	0.003
PBDE 028	0.339	0.743	A	0.105	0.305	B	0.046	0.161	B	0.104	0.354	B	0.001
PBDE 047	50.068	62.987	A	29.943	62.115	B	12.884	15.322	C	20.963	31.132	BC	0.000
PBDE 066	0.218	0.543	A	0.139	0.342	A	0.022	0.103	B	0.070	0.201	AB	0.000
PBDE 085	0.121	0.284	-	0.134	0.372	-	0.031	0.153	-	0.014	0.088	-	0.011
PBDE 099	27.351	44.471	A	16.147	36.268	AB	6.106	8.497	C	9.840	16.525	BC	0.000
PBDE 100	15.644	22.751	A	7.084	13.674	B	4.132	5.419	B	8.073	13.499	AB	0.000
PBDE 138	0.138	0.366	A	0.009	0.062	B	0.000	0.000	B	0.000	0.000	B	0.000
PBDE 153	6.093	7.602	A	3.215	8.114	B	1.336	2.142	B	2.502	5.565	B	0.000
PBDE 154	6.231	7.639	A	3.466	8.551	B	1.846	2.426	B	3.337	6.024	AB	0.000
SUMPBDE	106.554	140.702	A	60.326	129.061	B	26.482	32.752	C	45.060	71.501	BC	0.000
PCB 018	0.307	0.768	A	0.087	0.174	B	0.047	0.100	B	0.099	0.316	B	0.000
PCB 044	1.813	5.599	A	0.582	1.171	B	0.319	0.377	B	0.558	0.745	AB	0.000

Age	Immature			Subadult			Adult			Aged Adult			
	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	
N	34			44			111			38			P-value
PCB 049	2.450	6.563	A	0.986	2.351	AB	0.582	0.820	B	1.150	1.830	AB	0.005
PCB 052	4.731	11.251	A	2.170	4.163	B	1.025	1.238	B	1.540	2.638	B	0.000
PCB 056	0.050	0.139	A	0.037	0.084	AB	0.009	0.039	B	0.011	0.050	AB	0.012
PCB 087	6.413	11.992	A	3.968	8.037	AB	1.795	2.246	B	2.139	2.450	B	0.000
PCB 095	6.955	16.360	A	3.101	5.741	B	1.288	1.754	C	1.774	2.692	BC	0.000
PCB 097	4.056	10.393	A	2.426	7.857	AB	0.794	1.095	B	1.304	1.828	AB	0.001
PCB 099	17.100	31.024	A	13.056	42.792	AB	3.716	5.674	C	5.293	6.756	BC	0.000
PCB 101	20.388	40.784	A	11.644	25.704	AB	4.640	6.373	C	6.408	8.822	BC	0.000
PCB 105	7.213	9.775	A	6.402	17.968	AB	1.989	2.448	C	2.588	2.902	BC	0.000
PCB 110	6.027	10.305	A	4.227	9.244	AB	1.684	1.951	B	1.906	2.173	B	0.000
PCB 114	0.154	0.209	A	0.147	0.505	AB	0.043	0.100	B	0.071	0.135	AB	0.007
PCB 118	23.174	35.156	A	22.228	79.161	AB	6.541	8.112	C	9.696	10.769	BC	0.000
PCB 128	9.584	17.317	A	6.297	17.044	AB	2.119	3.082	C	3.447	5.127	BC	0.000
PCB 137	2.464	4.315	A	2.537	10.775	AB	0.530	0.756	C	0.921	1.352	BC	0.000
PCB 138	52.482	91.132	A	39.674	133.490	AB	11.471	13.872	C	20.547	31.193	BC	0.000
PCB 141	2.721	4.468	A	1.853	4.442	AB	0.600	1.009	C	0.820	1.294	BC	0.000
PCB 149	22.916	37.344	A	11.578	18.866	B	5.230	6.336	C	6.831	8.677	BC	0.000
PCB 151	6.736	12.403	A	3.373	4.624	AB	1.556	2.456	C	2.020	3.206	BC	0.000
PCB 153	76.482	140.207	A	45.926	127.382	B	13.483	18.369	C	21.418	33.403	BC	0.000
PCB 156	5.271	9.625	A	4.629	18.768	B	0.791	1.249	C	1.217	2.022	BC	0.000
PCB 157	1.350	2.464	A	1.125	4.426	AB	0.204	0.363	C	0.355	0.616	BC	0.000
PCB 158	4.409	9.232	A	3.785	14.221	AB	0.809	1.318	C	1.316	1.999	BC	0.000
PCB 170	8.741	13.497	A	5.348	12.698	B	2.042	3.287	C	2.868	4.374	BC	0.000
PCB 174	3.883	6.237	A	1.900	3.003	AB	1.059	1.798	B	1.385	2.361	B	0.000
PCB 177	8.106	14.330	A	3.593	4.283	AB	2.347	3.303	B	2.270	3.392	B	0.000
PCB 180	25.629	41.091	A	12.809	20.334	B	6.286	9.012	C	9.496	14.192	BC	0.000
PCB 183	6.843	11.568	A	3.746	6.217	AB	1.923	3.086	C	2.804	4.063	BC	0.000
PCB 187	30.040	49.073	A	14.624	12.227	A	11.246	12.948	B	10.051	13.345	B	0.000
PCB 189	0.492	0.936	A	0.280	0.895	AB	0.074	0.170	B	0.096	0.206	B	0.000
PCB 194	5.293	8.166	A	2.263	2.745	B	1.578	2.735	B	2.047	2.960	B	0.000

Age	Immature			Subadult			Adult			Aged Adult			
	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	
N	34			44			111			38			P-value
PCB 195	1.289	2.340	A	0.536	0.773	AB	0.647	3.744	B	0.345	0.656	B	0.000
PCB 200	1.139	2.063	A	0.499	0.560	AB	0.499	0.817	B	0.836	1.263	AB	0.014
PCB 201	7.710	10.661	A	3.271	3.434	B	2.526	3.924	B	2.549	3.640	B	0.000
PCB 203	4.994	7.539	A	2.216	2.318	B	1.618	2.824	B	1.829	2.599	B	0.000
PCB 206	2.561	5.071	A	0.994	1.646	B	0.983	2.203	B	1.028	1.445	AB	0.002
PCB 209	0.998	1.842	-	0.466	0.913	-	0.757	1.896	-	0.992	1.327	-	0.041
SUMPCB008-101	66.107	131.983	A	40.223	95.424	AB	16.084	19.709	C	23.115	28.129	BC	0.000
SUMPCB105-209	328.702	545.883	A	206.325	507.083	AB	80.632	101.372	C	111.751	154.344	BC	0.000
TOTALPCB	394.809	667.962	A	246.548	601.524	AB	96.716	119.397	C	134.866	180.216	BC	0.000

Pesticides

Univariate analyses

Based on univariate ANOVA results, in most cases, liver concentrations of most pesticides were greatest in immature otters and decreased with age (Figure 6). For example, cis- and trans-chlordane, DDD (p,p'), DDE (o,p'), and DDE (p,p') concentrations in liver were significantly greater in immature otters than in adults or aged adults, but were not significantly different from subadults (Tukey pairwise comparisons); the only exception was that immature and aged adult otters were not statistically different for DDD (p,p'). Concentrations of chlordane and DDT in subadult, adult, and aged adult sea otters were not significantly different between age classes, except that adult DDE (p,p') concentrations in liver were significantly lower than all other age classes. Dieldrin, HCH-beta, heptachlor epoxide, HCB, cis- and trans-nonachlor and oxychlordane were significantly more concentrated in livers of immature otters, when compared to subadults, adults, or aged adults. These same pesticides were less concentrated in adults than in subadults (with the exception of HCB) and were not significantly different in aged adults, when compared to adults or subadults (Figure 6). Generally, when stratified by age class, liver pesticide concentrations followed the schematic: immature>subadult>adult. Most pesticide concentrations then increased in aged adults, but not to the levels detected in immature animals.

Multivariate analyses

Based on ANCOVA analyses, significant interactions were detected between otter age and sex, affecting concentrations detected in otter livers for the following pesticides: sumDDT, sumChlordanes, dieldrin, and sumPCB ($P<0.05$). ANOVA analyses also revealed significant interactions between otter age class and sex, and between age and the stranding region for sumPBDE concentrations.

Multiple regression analyses revealed that age class was significantly and often negatively associated with hepatic concentrations of 14 of the 22 pesticides detected in sea otter livers (Table 5). Based on these results, immature otters were more likely to have elevated pesticide levels in liver tissue, with the exception of mirex, where aged adult otters were more likely to have higher liver levels. Subadult otters also had an increased risk of elevated levels of the following pesticides: p,p' DDD, o,p' DDE, p,p' DDE, dieldrin, beta-HCH, heptachlor epoxide, cis-nonachlor, trans-nonachlor, and oxychlordane, when compared to other age classes.

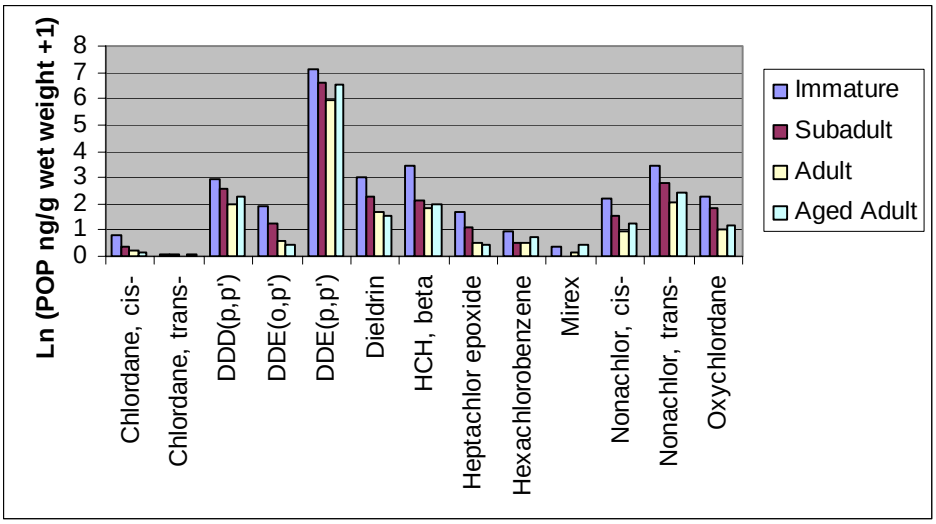

Figure 6. Concentrations of pesticides in southern sea otter liver samples showed statistically significant differences between age classes (ANOVA, P<0.05).

Organotins

Univariate analyses

Liver distributions of organotin POPs demonstrated a distinct concentration pattern, when stratified by sea otter age class, when compared to the other pesticide groups. In contrast to the detection of the highest liver pesticide concentrations in younger animals, as demonstrated by other POPs, livers from aged adult otters contained the highest concentrations of butyltins in the present study (Figure 7). Aged adult otter livers contained significantly higher concentrations of dibutyltin and summed butyltins than those of immature animals, and greater concentrations of tributyltin than both immatures and subadults. In fact, the mean butyltin load in livers from aged adults was seven times that of immature otters.

Multivariate analyses

Multiple regression analyses confirmed the direct associations between sea otter age class and liver butyltin concentrations for most organotins. Immature or subadult otters were less likely to have high hepatic loads of dibutyltin, tributyltin, and sumButyltins. Age was not a significant factor in determining the concentration of monobutyltin present in sea otter liver tissue (Table 5).

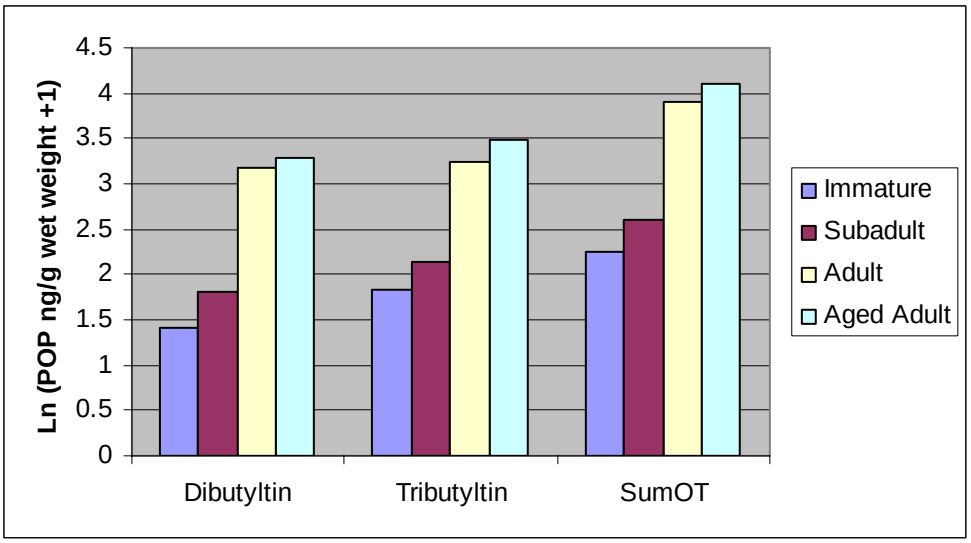

Figure 7. Associations between liver organotin concentrations and sea otter age class (ANOVA, P<0.05).

PAHs

Univariate analyses

On univariate ANOVA analyses, acenaphthylene was the only PAH that varied significantly by otter age class, using the following model: immatures < subadults < adults < aged adults. Livers from aged adult otters had significantly higher acenaphthylene concentrations than those of immature and subadults, but were not significantly different from adults. Aged adults had five times greater liver acenaphthylene concentrations than livers from immature otters.

Multivariate analyses

Based on multiple regression, increased otter age was a significant risk factor for detection of higher liver acenaphthylene concentrations, as was reported for the univariate analyses. Both adult and aged adult otters had a greater risk of having higher concentrations of acenaphthylene, when compared to immatures and subadults. Age was not a significant factor affecting other PAH concentrations detected in sea otter livers.

PBDEs

Univariate analyses

Based on single factor ANOVAs, hepatic concentrations of nine of the 14 PBDE congeners tested were significantly affected by age class (Figure 8). Immature otters had higher liver concentrations than subadults, adults, and aged adults for most PBDE congeners and sumPBDEs. However, immatures did not have significantly higher concentrations than subadults for PBDE congeners 66 and 99. Immature otters did have significantly higher PBDE concentrations than adults for all congeners. SumPBDE liver levels in immatures were higher than for all other age classes and levels in subadults were significantly higher than adults. In contrast, sumPBDE liver levels in immature, subadult and adult otters were not significantly different from those of aged adults (Table 6).

Multivariate analyses

Based on multifactor ANOVA, sea otter age class was inversely correlated with sumPBDE concentrations; however, significant age-sex and age-region interactions were also detected.

In multiple regression studies, immature and subadult otters were more likely to have high sumPBDEs in livers than adult or aged adult otters (Table 5). Multiple regression analyses were not conducted for individual analytes.

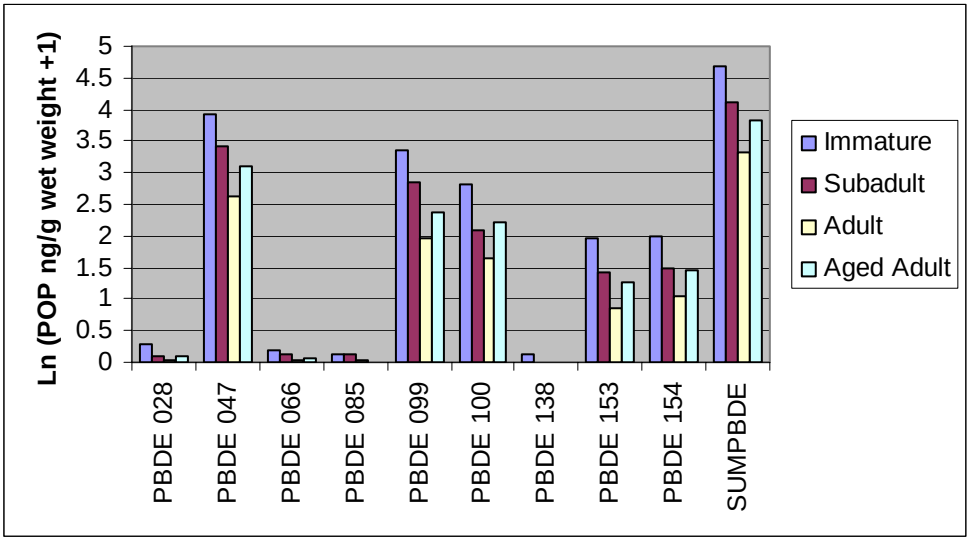

Figure 8. Liver concentrations of 9 of 14 PBDE congeners were significantly associated with sea otter age class. (ANOVA, P<0.05).

PCBs

Univariate analyses

Based on single factor ANOVAs, 38 of 48 PCB congeners examined in the present study varied significantly by sea otter age class. The overall pattern of PCB concentrations in liver by age class was that immature and subadult otters had significantly higher PCBs concentrations than adults, and aged adults did not differ significantly from adults (Figure 9 and Table 6). This overall pattern also held true for sumPCBs. In contrast, 11 of 19 congeners in the tetra-, penta-, hepta-, octa-, and nona- homolog groups did not differ significantly between adult and subadult otters. However, livers of adult otters had significantly lower levels of hexachlorinated PCBs than subadults, reflecting the same concentration pattern by age class demonstrated by sumPCBs.

Multivariate analyses

ANCOVA analyses for sumPCBs demonstrated significant interactions between sea otter age and sex, affecting sumPCB concentrations.

Multiple regression analyses for sumPCBs, sumPCB008-101, and sumPCB105-209 confirmed inverse associations between sea otter age class and liver PCB concentrations: Livers from immature and subadult otters were more likely to have high levels of PCB008-101 than adults and aged adults.

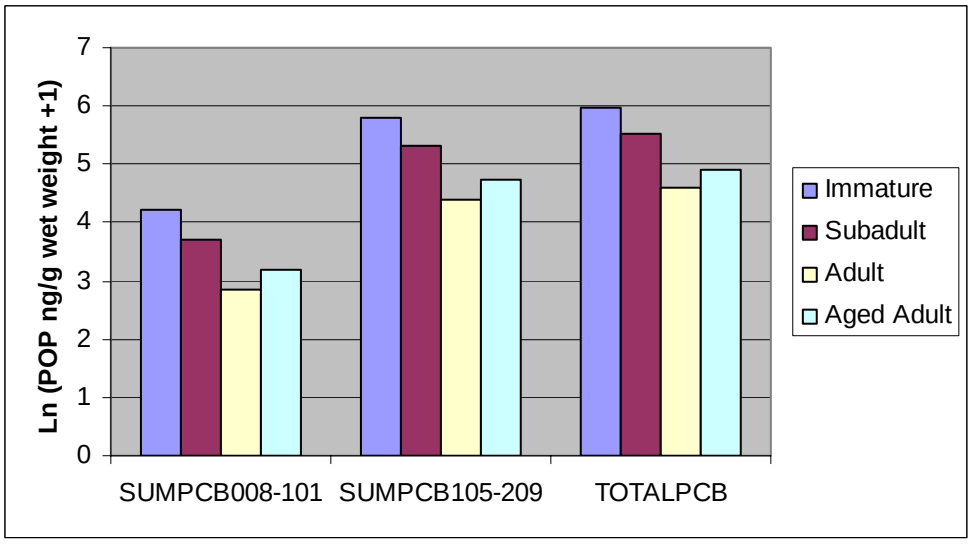

Figure 9. Associations between summed PCB concentrations in liver tissue and sea otter age class.

Body Condition

Univariate analyses

Concentrations of many POPs detected in sea otter livers varied significantly with nutritional condition (as determined by SQ fat scores) at the time of death. In univariate analyses a total of 60 POPs, including 10 of 29 pesticides, 1 of 47 PAHs, 0 of 3 butyltins, 9 of 14 PBDEs, and 40 of 47 PCBs analyzed varied significantly with nutritional condition. In the majority of cases, emaciated animals with no fat had the highest liver POP concentrations, and animals with abundant fat had the lowest concentrations (Table 7). This difference was significant for all POPs except chrysene and PCB 60 (Figure 10); chrysene was 8.7 times more concentrated in livers of animals that had abundant fat than in emaciated animals. PCB 60 was most concentrated in the livers of animals with scant fat, and these same animals had significantly higher PCB 60 levels than animals with abundant fat. Overall, the largest differences in liver POP concentrations were detected between sea otters with no SQ fat and those with abundant fat.

Multivariate analyses

Multifactor ANCOVA analyses conducted for sumPBDEs, sumDDT, sumChlordane, sumHCH, dieldrin and sumPCB body condition was a significant factor affecting the concentration of these POPs in sea otter tissue. In contrast, liver concentrations of HCB were not significantly affected by nutritional condition. No significant interactions were detected between SQ fat levels and sea otter age or sex.

Based on multiple regression analyses, having decreased body fat was a significant risk factor for detection of high hepatic POP concentration for 15 of the 25 POPs for which the analyses were conducted. Otters with no SQ fat at necropsy were most likely to have high liver levels of 12 of the 15 POPs affected by nutritional condition.

Table 7. POPs associated with the amount of subcutaneous (SQ) fat in sea otters. Reported P-values are the overall ANOVA result. SQ fat categories with the same letter in the Sig? column were not significantly different in subsequent pairwise analyses.

Fat	None			Scant			Fair			Moderate			Abundant			
	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	
N	77			56			17			34			43			P-value
Cis-chlordane	0.883	2.320	A	0.304	0.653	AB	0.388	0.643	AB	0.064	0.212	B	0.139	0.486	B	0.004
DDE(p,p')	1106.321	1254.918	A	611.865	1029.072	B	310.024	254.994	BC	257.391	215.255	BC	173.550	196.480	C	0.000
Dieldrin	11.661	16.081	A	6.910	7.748	AB	8.906	10.552	AB	2.486	2.007	B	2.863	3.374	B	0.000
HCH, beta	15.974	21.026	A	11.064	24.878	B	7.853	8.973	B	3.034	3.547	C	2.318	4.607	C	0.000
Heptachlor epoxide	2.630	2.924	A	1.599	1.590	AB	1.072	1.119	BC	0.518	0.637	C	0.390	0.664	C	0.000
Hexachlorobenzene	1.207	1.906	A	0.670	0.624	AB	0.944	0.896	AB	0.700	0.838	AB	0.501	0.530	B	0.021
Mirex	0.454	0.879	A	0.170	0.701	B	0.028	0.116	B	0.039	0.163	B	0.096	0.376	B	0.000
Cis-nonachlor	6.472	10.511	A	2.424	3.176	B	2.131	2.782	BC	0.560	1.017	CD	0.274	0.737	D	0.000
Trans-nonachlor	24.585	33.321	A	10.289	13.072	B	7.881	7.908	BC	3.798	3.784	CD	1.981	2.366	D	0.000
Oxychlordane	6.709	8.536	A	3.342	3.560	B	2.915	2.955	BC	1.131	1.308	CD	0.586	0.783	D	0.000
Chrysene	0.088	0.350	A	0.341	0.824	A	0.000	0.000	A	0.668	1.332	B	0.769	1.978	B	0.007
PBDE 017	0.166	0.452	-	0.081	0.308	-	0.022	0.091	-	0.000	0.000	-	0.031	0.121	-	0.044
PBDE 028	0.176	0.507	-	0.158	0.429	-	0.082	0.185	-	0.017	0.100	-	0.018	0.082	-	0.043
PBDE 047	35.426	40.267	A	31.207	64.979	AB	10.416	9.783	BC	8.638	6.543	C	6.983	7.357	C	0.000
PBDE 066	0.125	0.254	-	0.133	0.475	-	0.020	0.081	-	0.008	0.048	-	0.021	0.100	-	0.022
PBDE 085	0.093	0.241	-	0.114	0.353	-	0.000	0.000	-	0.014	0.080	-	0.000	0.000	-	0.025
PBDE 099	18.279	21.901	A	16.614	43.525	AB	4.927	4.251	BC	3.979	3.619	C	3.143	3.984	C	0.000
PBDE 100	12.116	15.453	A	8.199	16.787	B	2.990	4.313	BC	2.598	2.454	C	1.809	2.500	C	0.000
PBDE 153	4.659	5.064	A	3.233	8.785	B	1.038	1.177	BC	0.610	0.713	BC	0.320	0.620	C	0.000
PBDE 154	5.379	5.166	A	3.739	9.069	B	1.150	1.304	BC	0.815	0.841	C	0.588	0.926	C	0.000
SUMPBDE	76.609	84.845	A	63.515	141.880	B	20.643	19.408	BC	16.679	13.607	C	12.913	15.270	C	0.000
PCB 018	0.193	0.566	A	0.064	0.105	AB	0.106	0.249	AB	0.038	0.072	AB	0.039	0.073	B	0.034
PCB 044	1.231	3.836	A	0.434	0.477	AB	0.506	0.807	AB	0.231	0.223	B	0.195	0.206	B	0.000
PCB 049	1.995	4.794	A	0.716	1.046	B	0.725	1.101	AB	0.421	0.416	B	0.342	0.342	B	0.000
PCB 052	3.646	8.223	A	1.348	1.242	B	1.607	2.340	AB	0.689	0.514	B	0.505	0.397	C	0.000
PCB 060	0.093	0.160	AB	0.145	0.224	A	0.083	0.127	AB	0.063	0.104	AB	0.035	0.081	B	0.009
PCB 066	1.416	2.372	A	1.171	1.198	A	0.857	0.987	AB	0.650	0.562	AB	0.492	0.348	B	0.001

Fat	None			Scant			Fair			Moderate			Abundant			
	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	
N	77			56			17			34			43			P-value
PCB 074	0.762	1.611	A	0.632	0.831	A	0.446	0.640	AB	0.302	0.362	AB	0.166	0.233	B	0.000
PCB 087	5.452	9.958	A	2.266	2.335	B	2.250	3.988	B	1.286	1.119	B	1.036	0.799	B	0.000
PCB 095	5.033	11.798	A	1.799	1.728	B	2.184	3.470	AB	0.908	0.824	B	0.629	0.531	C	0.000
PCB 097	3.553	9.047	A	1.024	1.271	B	1.004	1.538	AB	0.556	0.580	B	0.360	0.372	B	0.000
PCB 099	15.998	37.937	A	5.648	6.726	B	4.889	9.286	BC	2.520	3.020	BC	1.222	0.973	C	0.000
PCB 101	16.935	33.263	A	6.537	7.596	B	5.608	9.027	BC	3.330	3.676	BC	1.986	1.732	C	0.000
PCB 105	7.225	14.895	A	3.033	3.011	B	2.449	2.857	BC	1.308	1.170	C	0.785	0.605	C	0.000
PCB 110	5.251	9.704	A	2.236	2.347	B	2.117	2.915	BC	1.284	1.134	BC	0.953	0.882	C	0.000
PCB 114	0.180	0.407	A	0.075	0.115	B	0.027	0.061	B	0.017	0.057	B	0.003	0.018	B	0.000
PCB 118	25.210	63.498	A	9.473	9.769	B	7.764	10.422	BC	4.423	4.409	C	2.476	1.684	C	0.000
PCB 128	8.787	17.113	A	3.113	4.157	B	2.486	3.829	BC	1.369	1.627	BC	0.682	0.623	C	0.000
PCB 137	2.849	8.548	A	0.740	1.020	B	0.536	0.651	BC	0.362	0.406	BC	0.162	0.167	C	0.000
PCB 138	51.376	116.783	A	16.996	20.068	B	13.105	17.860	BC	7.868	6.964	BC	4.327	3.195	C	0.000
PCB 141	2.513	4.471	A	0.836	1.110	B	0.555	0.804	BC	0.424	0.603	BC	0.178	0.227	C	0.000
PCB 149	17.527	28.881	A	7.436	7.587	B	5.693	8.758	BC	4.127	4.690	BC	2.920	2.800	C	0.000
PCB 151	5.281	9.102	A	2.148	2.426	B	2.134	4.086	BC	1.094	1.354	BC	0.616	0.672	C	0.000
PCB 153	65.175	133.582	A	20.597	22.030	B	16.088	23.600	B	9.068	9.668	BC	4.140	3.588	C	0.000
PCB 156	5.298	15.444	A	1.172	1.339	B	0.918	1.302	BC	0.540	0.780	BC	0.219	0.236	C	0.000
PCB 157	1.336	3.695	A	0.315	0.433	B	0.286	0.471	B	0.124	0.222	B	0.046	0.076	B	0.000
PCB 158	4.468	12.263	A	1.162	1.442	B	1.135	2.126	BC	0.488	0.624	BC	0.263	0.260	C	0.000
PCB 170	8.036	13.198	A	2.566	3.058	B	2.044	2.644	BC	1.338	1.833	BC	0.592	0.515	C	0.000
PCB 174	3.233	4.900	A	1.342	2.091	B	1.021	1.590	BC	0.792	1.072	BC	0.405	0.401	C	0.000
PCB 177	6.271	10.378	A	2.358	2.689	B	2.316	3.949	B	1.920	2.426	B	1.416	1.760	B	0.000
PCB 180	22.022	31.776	A	8.326	10.333	B	6.068	7.031	BC	4.287	5.766	BC	1.924	1.659	C	0.000
PCB 183	6.199	9.159	A	2.488	3.202	B	2.081	3.322	BC	1.215	1.611	BC	0.564	0.514	C	0.000
PCB 187	23.290	35.269	A	9.919	10.220	B	9.628	11.076	B	11.297	12.610	B	9.268	10.376	B	0.000
PCB 189	0.434	0.916	A	0.095	0.149	B	0.072	0.143	B	0.028	0.089	B	0.000	0.000	B	0.000
PCB 194	4.765	6.185	A	1.820	2.386	B	1.219	1.370	BC	0.804	1.151	BC	0.363	0.327	C	0.000
PCB 195	1.062	1.744	A	1.046	5.215	B	0.309	0.531	BC	0.123	0.255	BC	0.060	0.094	C	0.000
PCB 200	1.115	1.545	A	0.617	1.121	B	0.491	0.869	B	0.317	0.378	B	0.193	0.230	B	0.000

Fat	None			Scant			Fair			Moderate			Abundant			
	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	
N	77			56			17			34			43			P-value
PCB 201	6.493	8.331	A	2.403	2.938	B	2.315	2.780	B	1.774	2.037	B	1.142	1.136	B	0.000
PCB 203	4.442	5.867	A	1.639	2.112	B	1.522	1.800	BC	0.944	1.103	BC	0.570	0.432	C	0.000
PCB 206	2.497	4.057	A	0.880	1.722	B	0.781	1.098	BC	0.427	0.614	BC	0.223	0.218	C	0.000
PCB 209	1.511	2.390	A	0.609	1.349	B	0.458	0.663	B	0.288	0.395	B	0.188	0.207	B	0.000
SUMPCB008-101	56.889	112.939	A	22.231	22.133	B	20.659	30.793	BC	11.388	10.424	BC	7.381	5.353	C	0.000
SUMPCB105-209	293.844	525.865	A	105.439	113.042	B	85.617	115.968	BC	58.051	58.753	BC	34.676	28.680	C	0.000
TOTALPCB	350.733	632.614	A	127.670	133.595	B	106.276	146.121	BC	69.439	68.336	BC	42.057	33.316	C	0.000

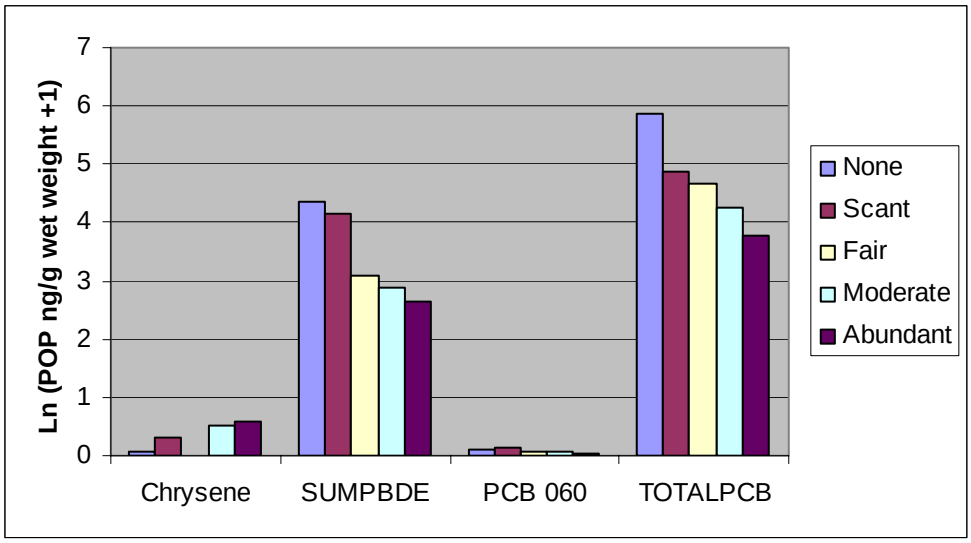

Figure 10. Liver concentrations of Chrysene are highest in animals with more SQ fat; the opposite pattern is demonstrated by sumPBDE and sumPCB concentrations.

Region

Overview

One-way single factor ANOVA (Table 8) and multiple regression (Table 5) results suggest that regional stranding locations were a significant factor affecting POP concentrations detected in sea otter livers. Nine of 29 pesticides, 1 of 47 PAHs, 6 of 14 PBDE congeners, 0 of 3 organotins, and 21 of 48 PCB congeners were significantly affected by regional stranding location, based on single factor ANOVAs.

Pesticides

Six of 9 pesticide compounds that were significantly affected by stranding region were higher in livers from sea otters that stranded within Monterey Bay, when compared to otters stranding in Estero Bay (Table 8). Levels of HCH, beta were significantly higher in sea otters recovered from the Big Sur region, when compared to otters stranding along the south coast. DDE (p,p') concentrations were higher in sea otters that stranding along the south coast, when compared to animals from the Big Sur region. DDE (p,p') levels were higher for sea otters stranding in Monterey Bay, when compared to sea otters from Big Sur. Monterey Bay sea otters also had higher levels of dieldrin and oxychlordane when compared to south coast sea otters; however no significant differences were detected between these regions for the other pesticides.

PAHs

Liver acenaphthylene concentrations were significantly higher in otters that stranded along the south coast, when compared to north coast otters. No other PAHs differed significantly between stranding locations.

PBDEs

Of six PBDE congeners (28, 47, 66, 85, 99 and 100) found to be significantly different between regions, all were detected at the highest concentrations in livers of sea otters stranding along the

south coast. All, except PBDE 100, were significantly higher than levels in otters stranding within Monterey Bay. PBDE 66 was also significantly more concentrated in south coast otters when compared to animals stranding in Estero Bay. PBDE congeners 47, 99, 100, and summed PBDEs were more concentrated in sea otters that stranded along the south coast, compared to sea otters that stranded along Big Sur. Based on univariate analyses, sea otters stranding in the south coast region had significantly higher concentrations of PBDEs than sea otters that stranding in Monterey Bay or Big Sur.

PCBs

Based on single factor ANOVAs, few significant differences were detected between regions when comparing the North Coast, Big Sur, and south coast regions to each other or to Monterey Bay and Estero Bay (Table 8).

Significant differences between regional stranding locations were detected for 21 of 48 examined PCB congeners. Twelve PCB congeners (49, 52, 87, 97, 151, 174, 177, 183, 200, 203, 206, and 209) were higher in sea otters stranding in Monterey Bay, when compared to those stranding in Estero Bay. Four additional PCB congeners (27, 66, 200, and 209) were higher in Monterey Bay otters, when compared to animals stranding along Big Sur. Overall, animals that stranding within Monterey Bay had higher liver concentrations of >50% of PCB congeners that were affected by otter stranding location than otters from Estero Bay.

Table 8. POPs in sea otter livers that were significantly different among regions. Reported P-value is overall ANOVA result - regions with the same letter in the Sig? column are not significantly different using subsequent pairwise analyses.

	North Coast			Monterey Bay			Big Sur			Estero Bay			South Coast			
	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	P-value
N	12			102			24			49			40			
cis-Chlordane	0.139	0.482	AB	0.516	1.041	A	0.973	3.369	AB	0.059	0.210	B	0.478	1.474	AB	0.033
DDD(o,p')	2.829	5.507	ABC	3.928	8.123	A	2.370	8.048	BC	0.212	0.562	C	3.191	5.342	AB	0.000
DDD(p,p')	8.018	14.007	AB	12.941	19.004	A	6.883	20.292	B	1.726	2.753	B	11.957	20.267	A	0.000
DDE(p,p')	517.167	460.927	AB	692.314	1042.421	A	647.371	1610.786	B	390.480	412.050	AB	736.075	895.836	A	0.010
Dieldrin	7.481	9.226	AB	8.666	10.222	A	11.553	22.678	AB	4.286	6.321	B	4.572	6.971	B	0.002
HCH, beta	9.991	8.879	AB	8.894	16.428	AB	20.887	39.879	A	8.034	9.744	AB	6.596	10.657	B	0.040
cis-Nonachlor	2.530	3.024	AB	4.005	6.830	A	4.365	14.211	AB	1.369	1.907	B	2.261	4.176	AB	0.011
trans-Nonachlor	11.924	13.316	AB	14.809	22.042	A	18.179	45.940	AB	7.198	8.751	B	9.372	14.655	AB	0.019
Oxychlordane	4.128	3.900	AB	4.576	6.872	A	4.085	8.508	AB	2.383	2.961	AB	2.146	3.668	B	0.005
Acenaphthylene	0.203	0.701	B	0.420	0.945	AB	1.141	1.773	AB	0.662	1.166	AB	1.359	1.771	A	0.001
PBDE 028	0.105	0.191	AB	0.021	0.106	B	0.162	0.697	AB	0.129	0.297	AB	0.289	0.573	A	0.001
PBDE 047	14.581	10.274	AB	17.128	22.237	B	20.023	43.039	B	19.096	22.773	AB	47.706	79.859	A	0.014
PBDE 066	0.050	0.119	AB	0.060	0.201	B	0.064	0.155	AB	0.038	0.116	B	0.212	0.563	A	0.049
PBDE 085	0.000	0.000	AB	0.028	0.125	B	0.027	0.134	AB	0.072	0.239	AB	0.175	0.410	A	0.011
PBDE 099	6.155	5.230	AB	7.642	11.353	B	8.080	15.432	B	11.170	16.847	AB	27.438	52.616	A	0.009
PBDE 100	3.970	3.722	AB	5.719	9.862	B	7.920	19.687	AB	4.736	4.895	AB	13.898	20.318	A	0.017
SUMPBDE	28.075	21.694	AB	35.588	50.913	AB	41.067	86.184	B	40.089	50.604	AB	100.304	172.227	A	0.032
PCB 027	0.000	0.000	AB	0.000	0.000	B	0.023	0.078	A	0.000	0.000	B	0.000	0.000	B	0.002
PCB 049	0.762	0.860	AB	1.006	1.457	A	2.227	7.809	AB	0.307	0.371	B	1.368	2.537	A	0.009
PCB 052	1.207	1.281	AB	1.890	2.481	A	3.699	13.378	AB	0.789	0.650	B	2.349	4.489	AB	0.044
PCB 066	0.808	0.651	AB	1.162	2.094	A	0.409	0.439	B	0.866	0.933	AB	1.299	1.228	A	0.006
PCB 074	0.371	0.441	AB	0.553	1.440	AB	0.188	0.353	B	0.494	0.606	AB	0.735	0.779	A	0.028
PCB 087	1.582	1.150	AB	3.076	4.159	A	3.947	12.569	AB	1.371	1.277	B	4.464	9.046	AB	0.007
PCB 097	0.928	0.829	AB	1.419	2.029	A	3.436	12.307	AB	0.525	0.525	B	2.957	8.235	A	0.028
PCB 101	5.040	3.770	-	8.649	13.329	-	13.058	44.394	-	4.103	4.355	-	12.674	28.229	-	0.044
PCB 110	2.531	2.347	-	2.728	3.688	-	3.541	11.320	-	1.433	1.422	-	4.660	9.689	-	0.029
PCB 138	15.201	11.764	-	24.602	42.565	-	30.014	85.402	-	11.710	11.791	-	39.955	141.004	-	0.033
PCB 151	1.873	1.453	AB	3.292	5.400	A	3.834	12.442	AB	1.365	1.529	B	2.743	4.995	AB	0.037
PCB 174	1.473	1.138	AB	2.043	3.312	A	1.721	5.005	AB	0.763	0.881	B	2.026	4.085	AB	0.016

	North Coast			Monterey Bay			Big Sur			Estero Bay			South Coast			
	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	Avg	SD	Sig?	P-value
N	12			102			24			49			40			
PCB 177	3.087	2.689	AB	4.270	7.264	A	4.139	12.100	AB	1.670	1.693	B	3.165	4.968	AB	0.013
PCB 180	10.061	7.820	-	13.449	24.483	-	12.062	26.882	-	6.288	7.017	-	10.087	21.416	-	0.038
PCB 183	2.562	2.084	AB	3.921	6.210	A	3.625	10.327	AB	1.574	1.686	B	3.069	6.529	AB	0.016
PCB 187	17.144	13.809	-	17.037	25.450	-	16.953	41.988	-	8.777	7.714	-	12.867	13.914	-	0.018
PCB 200	0.660	0.651	AB	0.925	1.309	A	0.619	1.977	B	0.287	0.280	B	0.416	0.547	B	0.000
PCB 201	3.358	2.146	-	4.354	6.764	-	3.704	7.881	-	2.097	2.230	-	2.681	4.077	-	0.042
PCB 203	1.805	1.398	AB	3.038	4.771	A	2.593	5.881	AB	1.256	1.318	B	1.526	2.283	AB	0.014
PCB 206	0.852	0.611	AB	1.837	3.528	A	1.201	3.379	AB	0.549	0.570	B	0.640	0.907	B	0.003
PCB 209	0.494	0.496	AB	1.299	2.227	A	0.507	1.470	B	0.226	0.276	B	0.360	0.415	B	0.000
SUMPCB008-101	17.047	12.002	AB	28.847	39.538	B	44.333	148.549	A	14.103	13.253	AB	44.479	102.197	AB	0.008
SUMPCB105-209	109.015	71.016	-	158.269	263.349	-	183.702	492.848	-	76.920	76.614	-	195.533	540.410	-	0.036
TOTALPCB	126.062	79.340	-	187.116	300.958	-	228.035	639.360	-	91.023	87.748	-	240.013	641.452	-	0.029

Associations of POP concentrations between sea otter tissues and mussels

Overview

Multifactor analysis of covariance (ANCOVA) was conducted to examine relationships between sea otter POP concentrations and mussel POP concentrations. The ANCOVA analyses included sex, age, and body condition as fixed categorical factors and mussel POP concentrations as a continuous covariate. ANCOVA analyses were conducted for the following POPs: sumDDT, sumChlordane, sumHCH, dieldrin, HCB, and sumPCBs. There were significant interactions between sex and age, and gender was a significant factor affecting liver sumDDT, sumChlordanes, dieldrin, and sumPCB concentrations ($P<0.05$). Multifactor ANCOVA analysis was not conducted for sumPBDE, because no PBDE data was available from the National Status and Trends Mussel Watch program. A separate multifactor ANOVA was conducted using sumPBDE as the dependent variable, and gender, age, body condition, and regional location as fixed categorical variables. The results of this multifactor ANOVA demonstrated significant interactions between gender and age, and between sex and coastal region in addition to the significant effect of sex as a single variable ($p<0.05$).

sumDDT

Significant interactions were detected between sea otter sex and mussel sumDDT concentrations ($p=0.0389$, df 1, 197, $F=4.3225$) (Figure 11). Using ordinary least squares regression analyses for sea otter Ln (sumDDT+1) versus mussel Log (sumDDT+1), no significant differences were detected for males, compared with significant positive correlations for females (adj. r^2=0.0887, y=3.8967+0.6696x, ANOVA p=0.0028, df 1, 86, F=9.4678). Based on ANCOVA analysis, highly significant interactions were also detected between otter age, sex and liver sumDDT concentrations ($p<0.0001$, df 3, 197, $F=5.0417$) (Figure 12). Highly significant associations were also detected between otter nutritional condition and sumDDT concentrations ($p=0.0000$, df 3, 197, $F=20.9328$).

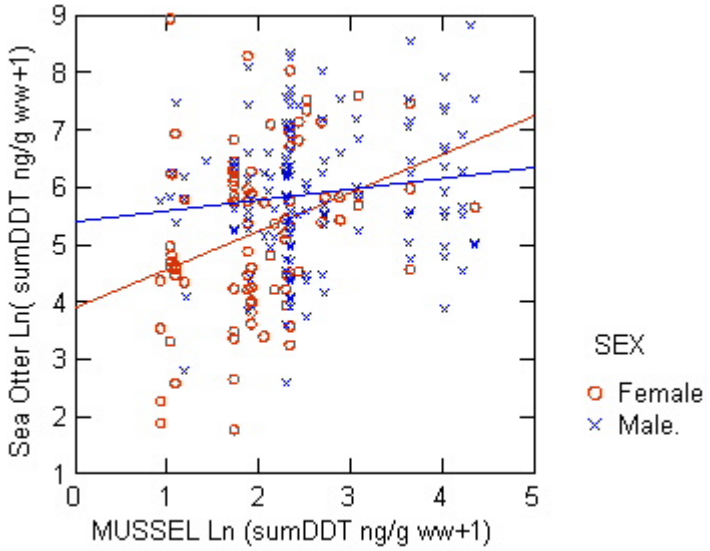

Figure 11. Comparison of sumDDT concentrations between mussels and southern sea otter livers, stratified by gender

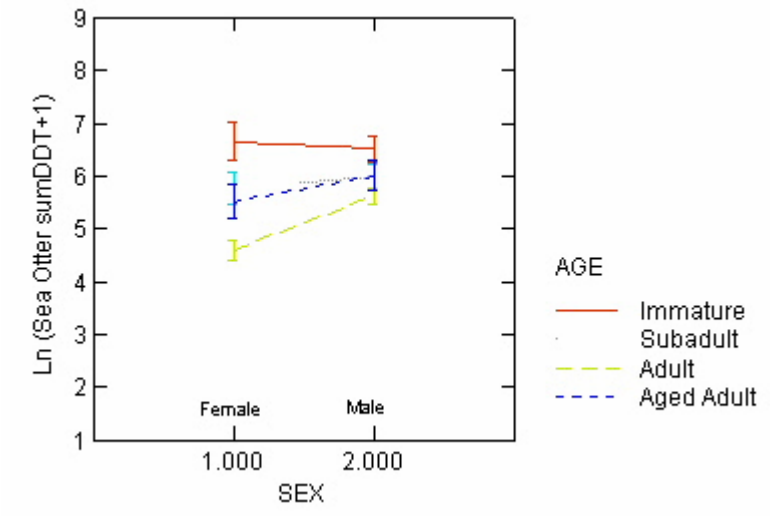

Figure 12. Comparison of Ln (sumDDT+1) levels in southern sea otters, stratified by gender and age class.

sumChlordane

No significant interactions were detected between sea otter age, sex, or body condition and mussel sumChlordane concentrations. Significant interactions were detected between sea otter sex and age (p=0.0099, df 1, 198, F=3.8927). Highly significant associations were detected between mussel sumChlordane concentrations and those in sea otter livers (p=0.0000, df 1, 198, F=27.5395) (Figure 13). Nutritional condition was also highly significant with respect to liver sumChlordane concentrations in sea otters (p=0.0000, df 3, 198, F=19.9758).

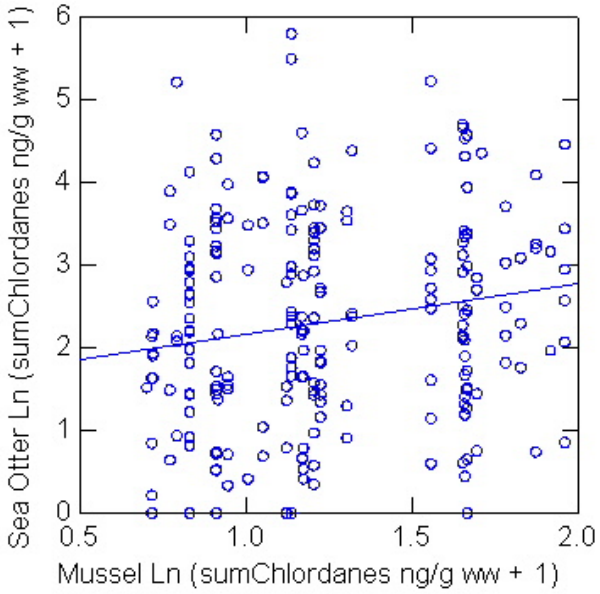

Figure 13. OLS regression analysis of sea otter versus mussel Log (sumChlordanes ng/g ww + 1) (adj. r^2=0.0271, y=1.5411+0.6187x, ANOVA p=0.0081, df 1, 220, F=7.1457).

sumHCH
No significant interactions were detected between sea otter age, sex, or nutritional condition and mussel sumHCH concentrations. Significant factors for sumHCH concentrations in sea otter livers included sea otter age class (p=0.0000, df 3, 198, F=12.3443) and nutritional condition (p=0.000, df 3, 198, F=15.5479).

Dieldrin
No significant interactions between age, sex, or nutritional condition were detected for mussel dieldrin concentrations. Significant interactions were detected between sea otter age and sex (p=0.0000, df 3, 198, F= 4.8513). Highly significant positive associations were detected between mussel dieldrin concentrations and liver dieldrin concentrations in sea otters (p=0.0000, df 1, 198, F= 29.8026) (Figure 14). Highly significant positive associations were also detected between levels of SQ fat in otters and liver dieldrin concentrations (p=0.0002, df 3, 198, F=6.9181).

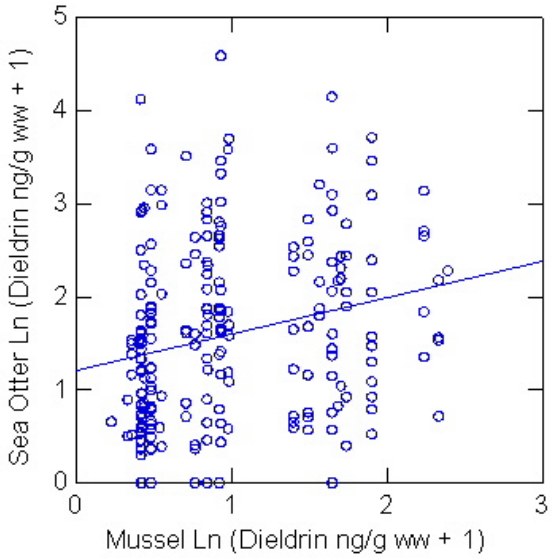

Figure 14. OLS regression analysis of sea otter versus mussel Ln (sumDieldrin ng/g ww + 1) (adj. r^2=0.0496, y=1.2014+0.3965x, ANOVA p=0.0005, df 1, 220, F=12.5229).

HCB
No significant interactions were detected for otter age class, gender or body condition and sumPCB concentrations. Sea otter age was associated with liver HCB concentrations (p=0.0082, df 3, 198, F= 4.0372).

sumPCBs
Interactions between otter age class, gender and body condition were not detected for mussel sumPCB concentrations. Significant interactions were detected between sea otter age class and sex (p=0.0107, df 3, 198, F= 3.8323). Mussel sumPCB concentrations were positively correlated

with liver sumPCB concentrations in sea otters (p=0.0329, df 1, 198, F= 4.6170). Nutritional condition was also associated with liver sumPCB concentrations (p=0.0000, df 1, 198, F= 15.

Associations of POP test results with otter proximity to freshwater runoff and municipal wastewater effluent

The majority of sea otters in our sample population stranded in locations characterized by low exposure to freshwater flows and municipal wastewater discharges (Table 9). Slight differences were detected in the percentage of male and female otters exposed to each of these potential sources of contaminants and pathogens, with a higher percentage of males experiencing moderate exposure to freshwater flows and wastewater, when compared to females (Figure 15). Gender differences in exposure to freshwater flows and wastewater are evident across all age groups, with the exception of subadults (Figure 16). Immature, adult and aged adult males had slightly higher proportions of individuals with moderate exposure to freshwater flows and wastewater than did females of similar age classes. Aged adult males had the greatest proportion of individuals exposed to high freshwater runoff; in contrast, aged adult females had the lowest exposure to high freshwater runoff.

Table 9. Distribution of southern sea otters by gender and region from stranding locations exposed to various levels of coastal freshwater and wastewater discharges.

Gender	Freshwater Flows			Wastewater	
Region	Low	Moderate	High	Low	Moderate
Male					
North Coast	6	0	0	6	0
Monterey Bay	27	27	11	57	8
Big Sur	3	2	0	2	3
Estero Bay	15	11	3	16	13
South Coast	17	14	2	12	21
All Regions	68	54	16	93	45
Female					
North Coast	5	0	1	5	1
Monterey Bay	27	7	3	37	0
Big Sur	9	8	2	11	8
Estero Bay	11	5	4	14	6
South Coast	6	1	0	4	3
All Regions	58	21	10	71	18
Grand Total	126	75	26	164	63

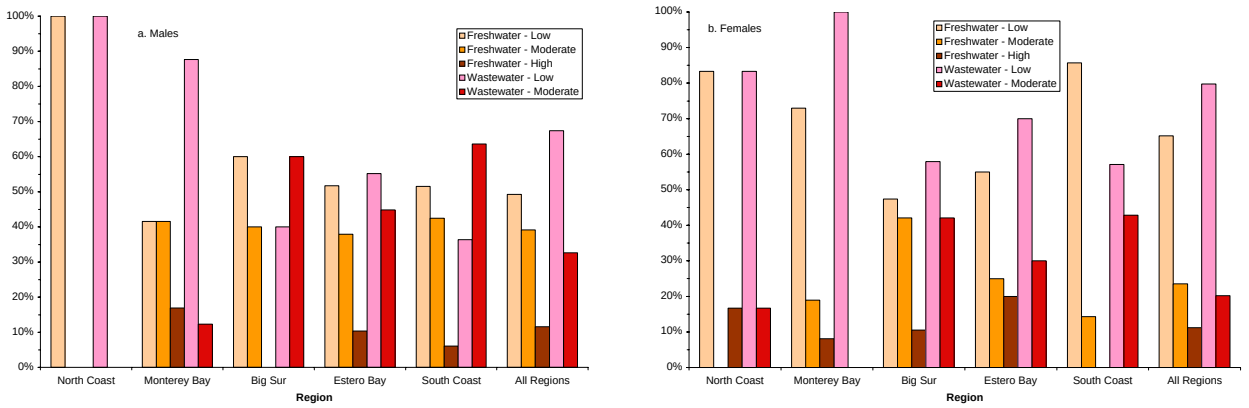

Figure 15. Percentage of male and female sea otters stranding by region, stratified by exposure to coastal runoff and wastewater outflows.

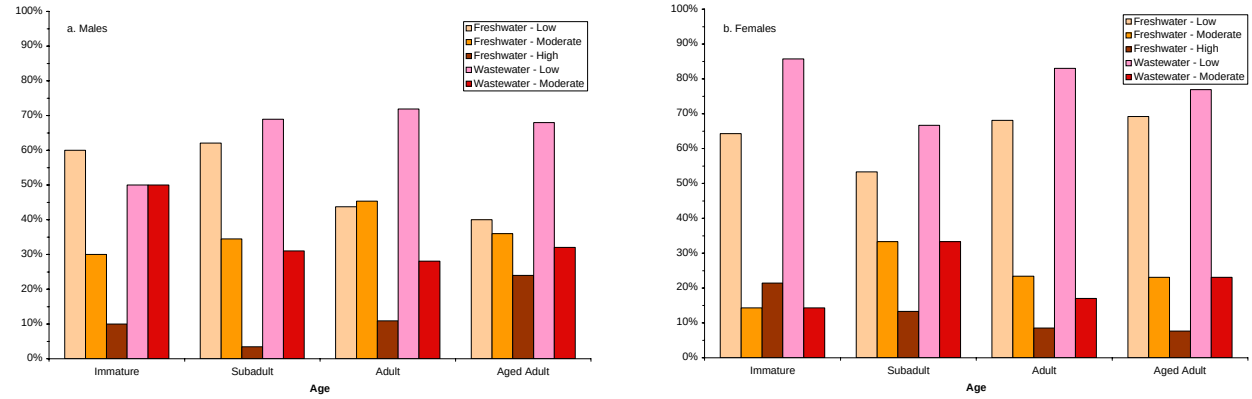

Figure 16. Percentage of male and female sea otters stranding by age class, stratified by exposure to coastal runoff and wastewater outflows.

Several contaminants varied in sea otter livers according to the animals' exposure to freshwater flows (Table 10), though only one, [DDD (o,p')], showed statistically significant associations (using ANOVA methods). The highest liver levels of this compound were detected in otters stranding in areas of moderate freshwater outflow; these findings were significantly different for otters stranding in areas of low freshwater outflow. Interestingly, otters exposed to high freshwater flows, based on the stranding location, exhibited intermediate DDD (o,p') concentrations in the liver; these findings were not significantly different from otters stranding in low or moderate outflow areas. Five additional POPs [DDD (p,p'); DDE (o,p'); HCH, delta; dibenzothiophene, C2; and PBDE 17] exhibited statistically significant pairwise results, though subsequent ANOVA showed no overall differences (data not shown). Sea otter liver

concentrations of DDD, DDE and PBDE were higher in animals stranding near moderate freshwater outflow areas, when compared with animals exposed to low freshwater outflows, while HCH and PAH analytes were highest in livers of otters from high flow areas as compared with animals exposed to low coastal freshwater flow.

Some contaminants also varied in sea otter livers according to the relative animal exposure to municipal wastewater effluent, based on each animal's stranding location (Table 11). Six analytes: [DDE (o,p'); dibenzothiophene, C2-; and 4 PBDEs: 28, 66, 85 and 99] exhibited higher concentrations in sea otters stranding in areas exposed to moderate wastewater outflows; liver burdens of the above analytes in otter livers increased proportionally with moderate exposure to municipal wastewater effluent discharges. No otters in the current study were recovered from areas of heavy exposure to municipal wastewater. Two of these same compounds (DDE(o,p') and C2-Dibenzothiophene) were also detected at higher concentrations in otters stranding in areas with heavy exposure to freshwater runoff, as described above. In contrast, liver concentrations of PCB 200 and PCB 209 varied inversely with wastewater exposure.

Associations of POP test results in sea otters with coastal human population density

Most of the sea otters examined stranded in areas with human population density ranging between 101–3,000 persons/mile^2 (Table 12). This pattern was consistent among sexes and ages of stranding otters. When compared to the long stretches of shoreline adjacent to areas with <100 persons/mile^2, this stranding pattern suggests that stranded otters are more likely to be recovered in areas with greater human population density.

Single factor ANOVA analyses with human population density as the independent categorical variable showed that variations in coastal population density were significantly associated with liver concentrations of only four POPs: dibutyltin, sumOT, acenaphthylene, and PBDE 183. Dibutyltin and sumOT were lowest in areas with human population densities of 100-1000 persons/mile^2; this difference was statistically significant only for sumOT between areas with 101-1000 persons/mile^2 and those with 3001-6000 persons/mile^2 (Table 13).

Results of multiple regression analyses confirmed that coastal human population density was a significant factor affecting liver concentrations of several POPs in otters stranding just offshore, including dieldrin, heptachlor epoxide, cis-nonachlor, trans-nonachlor, oxychlordane, dibutyltin, tributyltin, sumOT, biphenyl, sumPBDE, sumPCB008-101, sumPCB105-209, and totalPCB (Table 5). Otters stranding in areas with population densities >3000 persons/mile^2 generally had an increased risk of having higher liver concentrations of POPs than those stranding in areas with less dense coastal human populations.

Crude and adjusted associations of POP test results with major findings at necropsy

Overview

Risk factors with a P value <0.05 and odds ratios >1 significantly enhance an animal's risk of having the condition in question, while odds ratios <1 decrease the overall risk (i.e., are "protective"). Crude odds ratios are not corrected for possible contribution to the degree of risk measured by other factors, such as having a large number of otters in poor nutritional condition or of a young age class in the sample population; adjusted odds ratios have been corrected for these interactions and are a closer approximation of the degree of increased or decreased risk contributed by the named attribute, such as Dibenzothiophenes, C1- in Table 14 below.

Trauma

Univariate associations for liver POPs and other risk factors in relation to death due to trauma are summarized in Table 14. Otters dying with significant trauma were nearly twice as likely to be in good nutritional condition as otters dying without trauma. Since many sources of trauma result in acute death, this finding is not surprising. Adult otters were 2.5 times more likely to strand with trauma than other age classes. In addition, otters stranding along the coast north of Santa Cruz (ATOS 1-246) were 4.5 times more likely to die with significant trauma, especially trauma due to shark predation. The north coast has been reported to be a high-risk area for otter death due to predation in previous studies (Ames and Morejohn, 1980; Ames et al., 1996; Kreuder et al., 2003). Conversely, otters stranding within Monterey Bay were half as likely to have trauma as a significant finding as otters in other regions.

For the various POPs tested, when analyzed using univariate techniques, many were found to be significant risk factors for the finding of trauma during necropsy – some being "protective" (with crude odds ratios <1.0) and some associated with increased risk (crude odds >1.0). However, when these factors were corrected for variation due to otter age class, sex, location and nutritional condition, only C1-dibenzothiophene was associated with a significantly increased risk of trauma. Interestingly, in the crude odds, a number of pollutants showed statistically significant "protective" effects, especially heptachlor epoxide and oxychlordane. However, when adjusted for demographic and environmental risk factors, these findings became insignificant.

Table 10. Mean concentrations of POP analytes in sea otter livers that differed significantly with respect to otter proximity to major coastal points of freshwater runoff.

	Low			Moderate			High			Results of pairwise tests		
	Mean	SD	Sig?	Mean	SD	Sig?	Mean	SD	Sig?	T-Test	T-Test	T-Test
N	126			75			26			1v2	1v3	2v3
DDD(o,p')	1.822	4.578	B	4.447	9.344	A	2.557	5.208	AB	0.005	0.495	0.289
DDD(p,p')	6.796	11.607	-	14.534	24.869	-	7.610	11.949	-	0.048	0.615	0.443
DDE(o,p')	1.202	2.900	-	3.076	9.092	-	1.055	1.912	-	0.039	0.847	0.291
HCH, delta	0.000	0.000	-	0.004	0.027	-	0.013	0.054	-	0.217	0.045	0.392
Dibenzothiphene C2	0.027	0.304	-	0.017	0.145	-	0.161	0.569	-	0.957	0.049	0.060
PBDE 017	0.048	0.204	-	0.160	0.467	—-	0.037	0.131	—-	0.030	0.864	0.219

Table 11. Mean concentrations of POP analytes in sea otter livers that differed significantly with respect to otter proximity to major municipal wastewater outfalls.

	Low		Moderate		P-Value
	Mean	SD	Mean	SD	
N	164		63		
DDE(o,p')	1.479	5.728	2.651	5.723	0.043
Dibenzothiphene C2	0.013	0.162	0.109	0.525	0.035
PBDE 028	0.075	0.328	0.204	0.468	0.006
PBDE 066	0.052	0.166	0.160	0.468	0.013
PBDE 085	0.032	0.137	0.140	0.368	0.002
PBDE 099	8.312	13.365	21.094	43.277	0.016
PCB 200	0.733	1.283	0.437	0.661	0.046
PCB 209	0.933	1.878	0.367	0.651	0.003

Table 12. Number of sea otters stranding in areas with different levels of human population density.

	Human Population Density, persons/mile^2				
Population	0-100	101-1000	1001-3000	3001-6000	>6000
Male					
Immature	2	7	7	2	2
Subadult	6	6	12	4	1
Adult	12	19	18	8	7
Aged Adult	5	11	4	3	2
All Ages	25	43	41	17	12
Female					
Immature	2	5	2	2	3
Subadult	3	5	5	2	0
Adult	11	10	17	2	7
Aged Adult	1	1	5	0	6
All Ages	17	21	29	6	16
Grand Total	42	64	70	23	28

Table 13. Univariate ANOVA results show that variations in coastal human population density near stranding areas affected sea otter liver concentrations of the following POPs: dibutyltin, sumOTs, acenaphthylene, and PBDE 183.

Population	<100/mile^2			100-1000/mile^2			1000-3000/mile^2			3000-6000/mile^2			>6000/mile^2		
	Avg	StDev	Sig?	Avg	StDev	Sig?	Avg	StDev	Sig?	Avg	StDev	Sig?	Avg	StDev	Sig?
N	42			64			70			23			28		
Dibutyltin	27.405	135.689	-	3.613	8.680	-	17.734	35.390	-	8.790	16.410	-	35.159	95.724	-
SumOT	52.875	237.488	AB	8.741	17.624	B	39.557	81.508	AB	19.272	32.226	A	85.111	207.326	AB
Acenaphthylene	0.119	0.435	AB	0.033	0.265	B	0.105	0.561	A	0.000	0.000	AB	0.060	0.319	AB
PBDE 183	0.000	0.000	B	0.010	0.080	AB	0.041	0.339	B	0.199	0.955	A	0.124	0.383	B

Table 14. Univariate model, showing associations between liver POP concentrations in sea otters and death with significant trauma.

	Chi 2 P-Value	Students T P-Value	Crude OR	95% Upper	95% Lower	Adjusted OR	95% Upper	95% Lower
N Coast	0.020		4.500	17.097	1.184			
Monterey Bay	0.040		0.566	0.970	0.330			
Adult	0.000		2.521	4.335	1.466			
Moderate Fat	0.027		1.867	3.259	1.070			
DDE(p,p')		0.005	1.000	1.000	1.000	1.000	1.000	1.000
Dieldrin		0.001	0.937	0.977	0.898	0.961	1.007	0.916
HCH, beta		0.041	0.958	0.991	0.926	0.970	1.011	0.931
Heptachlor epoxide		0.010	0.781	0.928	0.657	0.895	1.124	0.713
cis-Nonachlor		0.007	0.943	1.005	0.884	0.988	1.049	0.931
trans-Nonachlor		0.007	0.984	1.001	0.968	0.997	1.015	0.980
Oxychlordane		0.002	0.896	0.968	0.830	0.939	1.031	0.856
Acenaphthylene		0.036	2.421	6.368	0.920	1.935	5.377	0.696
Dibenzothiophenes, C1-		0.002	2.924	6.685	1.279	2.953	7.174	1.216
Naphthalenes, C4 -		0.005	N/A	N/A	N/A	N/A	N/A	N/A
PCB_052		0.018	0.903	1.022	0.798	0.967	1.070	0.874
PCB_087		0.018	0.946	1.015	0.882	0.981	1.043	0.923
PCB_095		0.009	0.927	1.014	0.848	0.974	1.050	0.903
PCB_099		0.000	0.961	0.999	0.924	0.985	1.015	0.956
PCB_101		0.005	0.977	1.004	0.951	0.990	1.012	0.969
PCB_105		0.000	0.915	0.993	0.843	0.970	1.034	0.910
PCB_110		0.026	0.954	1.017	0.895	0.985	1.044	0.929
PCB_118		0.000	0.973	0.999	0.948	0.991	1.012	0.971
PCB_128		0.001	0.946	1.004	0.891	0.977	1.022	0.933
PCB_137		0.007	0.817	1.026	0.651	0.937	1.112	0.789
PCB_138		0.000	0.988	1.001	0.976	0.996	1.005	0.987
PCB_141		0.015	0.870	1.022	0.741	0.943	1.084	0.820
PCB_149		0.014	0.980	1.004	0.958	0.995	1.016	0.973
PCB_151		0.003	0.914	0.997	0.838	0.961	1.047	0.881
PCB_153		0.001	0.989	1.000	0.978	0.995	1.004	0.985
PCB_156		0.003	0.871	1.006	0.753	0.943	1.070	0.831
PCB_157		0.011	0.654	1.043	0.410	0.850	1.224	0.590
PCB_158		0.003	0.892	1.022	0.779	0.957	1.055	0.868
PCB_170		0.003	0.930	0.995	0.870	0.962	1.026	0.903
PCB_174		0.040	0.933	1.030	0.845	1.006	1.121	0.903
PCB_177		0.041	0.949	1.013	0.890	0.983	1.049	0.921
PCB_180		0.004	0.974	0.998	0.952	0.985	1.010	0.961
PCB_183		0.003	0.927	0.998	0.860	0.961	1.036	0.891
PCB_189		0.034	0.350	1.127	0.109	0.589	1.593	0.218
PCB_194		0.014	0.911	1.001	0.829	0.956	1.065	0.858
PCB_195		0.018	0.701	1.035	0.475	0.852	1.215	0.597
PCB_203		0.038	0.925	1.015	0.843	0.974	1.080	0.879
PCB_206		0.040	0.912	1.041	0.799	0.966	1.114	0.837
SUMPCB008-101		0.002	0.992	1.001	0.983	0.997	1.004	0.990
SUMPCB105-209		0.003	0.998	1.000	0.996	0.999	1.001	0.998
TOTALPCB		0.002	0.999	1.000	0.997	0.999	1.001	0.998

Bacterial disease

Univariate associations between liver POP concentrations and other risk factors in relation to death due to bacterial disease are summarized in Table 15. Demographic and spatial factors associated with increased risk for death due to bacterial infection included otters stranding within Monterey Bay, animals with scant fat at the time of necropsy and those that stranded as aged adults (1.7, 2.5 and 2.6 times increased risk, respectively). A decreased risk of death with significant bacterial disease was identified for otters with moderate body fat and for immature animals (both approximately 2.6 times decreased risk). For the various POPs tested, as was seen in trauma, several pollutants appeared significant when analyzed alone, but only PCB 56 proved statistically significant when adjusted for all demographic and spatial risk factors. While on the surface an odds ratio of over 700 appears extraordinarily high, it should be noted that this is the increased odds was associated with a 1.0 unit increase in liver PCB burden. A more realistic increase in PCB levels (0.1 ng/g) would provide an adjusted odds ratio of 1.95 – still high but not unrealistically so.

Table 15. Univariate model, showing associations between liver POP concentrations in sea otters and death due to bacterial disease.

	Chi 2 P-Value	Students T P-Value	Crude OR	95% Upper	95% Lower	Adjusted OR	95% Upper	95% Lower
Monterey Bay	0.045		1.719	2.918	1.012			
Immature	0.010		0.376	0.815	0.174			
Aged	0.010		2.588	5.516	1.214			
Scant Fat	0.002		2.45	4.386	1.369			
Moderate Fat	0.001		0.37	0.654	0.21			
PCB 044		0.031	1.375	2.002	0.945	1.341	2.055	0.875
PCB 049		0.020	1.222	1.501	0.995	1.199	1.504	0.956
PCB 056		0.016	378.245	92279.492	1.550	787.961	253230.682	2.452
PCB 060		0.042	6.154	38.280	0.989	3.535	28.574	0.437
PCB 066		0.014	1.446	1.947	1.073	1.284	1.819	0.906
PCB 070		0.048	4.043	14.397	1.135	3.732	15.709	0.886
PCB 074		0.042	1.536	2.371	0.995	1.303	2.147	0.791

Acanthocephalan peritonitis

Univariate associations for liver POPs and other risk factors in relation to death due to acanthocephalan peritonitis are summarized in Table 16. Similar to findings in previous studies (Mayer et al., 2003; Kreuder et al., 2003) immature otters were more likely to die with significant acanthocephalan peritonitis; in the present study the level of risk was almost 4 times higher for immature otters than for all other examined age classes. With respect to POPs, when analyzed alone, the risk of dying with moderate to severe acanthocephalan peritonitis was slightly increased for otters with higher liver levels of DDD (p,p'), dieldrin, heptachlor epoxide, cis-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, and PBDE 128. However, none of these compounds were significant when adjusted for age, sex, location and fat.

A decreased risk of death with moderate to severe acanthocephalan peritonitis was noted for adult otters and those with moderate subcutaneous fat (2.5 times and 3.4 times less risk, respectively). Interestingly, otters dying with acanthocephalan peritonitis were >20 times less likely to strand in areas exposed to higher levels of municipal wastewater effluent than other areas. In addition, animals with higher levels of PCBs 28 and 31 were significantly less likely to die due to

acanthocephalan peritonitis, even when corrected for contribution of risk by various demographic and environmental factors.

Table 16. Univariate model, showing associations between liver POP concentrations in sea otters and death with moderate to severe acanthocephalan peritonitis

	Chi 2 P-Value	Students T P-Value	Crude OR	95% Upper	95% Lower	Adjusted OR	95% Upper	95% Lower
Immature	0.000		3.949	8.431	1.849			
Adult	0.000		0.397	0.751	0.210			
Moderate Fat	0.001		0.290	0.630	0.133			
Low wastewater exposure	0.000		0.049	0.206	0.011			
cis-Chlordane		0.004	1.445	1.937	1.078	1.284	1.719	0.960
DDD(p,p')		0.037	1.016	1.032	1.001	1.005	1.023	0.986
DDE(p,p')		0.007	1.000	1.000	1.000	1.000	1.000	1.000
Dieldrin		0.000	1.054	1.087	1.022	1.030	1.065	0.996
HCH, beta		0.009	1.008	1.023	0.994	0.991	1.013	0.970
Heptachlor epoxide		0.000	1.242	1.436	1.074	1.037	1.253	0.858
cis-Nonachlor		0.001	1.067	1.125	1.012	1.028	1.079	0.978
trans-Nonachlor		0.001	1.015	1.029	1.002	1.004	1.019	0.990
Oxychlordane		0.000	1.124	1.200	1.053	1.074	1.158	0.996
PBDE_047		0.004	1.005	1.012	0.999	1.000	1.009	0.992
PBDE_066		0.017	2.125	5.782	0.781	1.304	3.810	0.446
PBDE_099		0.002	1.008	1.019	0.998	1.001	1.014	0.989
PBDE_100		0.011	1.019	1.040	0.998	1.007	1.033	0.981
PBDE_138		0.007	13.222	162.056	1.079	5.799	69.633	0.483
PBDE_153		0.002	1.055	1.112	1.000	1.019	1.078	0.964
PBDE_154		0.017	1.047	1.101	0.996	1.014	1.071	0.959
SUMPBDE		0.003	1.003	1.006	1.000	1.000	1.004	0.997
PCB_028		0.022	0.019	0.414	0.001	0.001	0.051	0.000
PCB_031		0.033	0.000	0.618	0.000	0.000	0.334	0.000
PCB_052		0.005	1.068	1.162	0.982	1.034	1.105	0.968
PCB_087		0.005	1.037	1.084	0.992	1.014	1.065	0.965
PCB_095		0.002	1.052	1.118	0.991	1.026	1.076	0.978
PCB_099		0.000	1.007	1.019	0.995	1.002	1.015	0.989
PCB_101		0.005	1.011	1.025	0.997	1.004	1.019	0.990
PCB_105		0.000	1.019	1.051	0.987	1.003	1.039	0.969
PCB_110		0.003	1.042	1.092	0.995	1.019	1.072	0.968
PCB_118		0.000	1.002	1.009	0.995	0.999	1.009	0.990
PCB_128		0.001	1.019	1.046	0.993	1.006	1.035	0.977
PCB_137		0.015	1.010	1.065	0.959	0.991	1.067	0.920
PCB_138		0.000	1.002	1.006	0.998	1.000	1.005	0.996
PCB_141		0.014	1.063	1.170	0.967	0.995	1.121	0.884
PCB_149		0.006	1.014	1.031	0.998	1.004	1.021	0.987
PCB_151		0.000	1.070	1.138	1.006	1.033	1.094	0.975
PCB_153		0.000	1.003	1.006	0.999	1.001	1.005	0.998
PCB_156		0.002	1.010	1.039	0.981	0.999	1.036	0.964
PCB_157		0.029	1.042	1.174	0.924	0.993	1.157	0.852
PCB_158		0.001	1.017	1.055	0.981	1.004	1.047	0.963

	Chi 2 P-Value	Students T P-Value	Crude OR	95% Upper	95% Lower	Adjusted OR	95% Upper	95% Lower
PCB_170		0.001	1.026	1.061	0.992	1.004	1.043	0.965
PCB_174		0.024	1.063	1.155	0.979	0.993	1.095	0.901
PCB_177		0.001	1.055	1.111	1.002	1.025	1.079	0.974
PCB_180		0.001	1.015	1.030	1.000	1.005	1.021	0.989
PCB_183		0.001	1.051	1.103	1.001	1.019	1.075	0.967
PCB_187		0.016	1.012	1.026	0.999	1.006	1.021	0.992
PCB_189		0.040	1.394	2.263	0.859	1.076	1.878	0.617
PCB_194		0.002	1.082	1.165	1.005	1.025	1.111	0.946
PCB_195		0.000	1.434	1.977	1.040	1.221	1.609	0.927
PCB_200		0.025	1.229	1.563	0.967	1.081	1.417	0.825
PCB_201		0.015	1.047	1.102	0.995	1.006	1.066	0.949
PCB_203		0.004	1.082	1.168	1.003	1.027	1.117	0.943
PCB_206		0.003	1.120	1.251	1.003	1.059	1.198	0.936
SUMPCB008-101		0.002	1.003	1.007	0.999	1.001	1.006	0.997
SUMPCB105-209		0.000	1.001	1.002	1.000	1.000	1.001	0.999
TOTALPCB		0.000	1.001	1.001	1.000	1.000	1.001	0.999

Protozoal disease or meningoencephalitis

Univariate associations for liver POPs and other risk factors in relation to death due to systemic or brain-based protozoal disease are summarized in Table 17. Similar to prior studies (Miller et al., 2002b), otters stranding near areas of moderate to heavy coastal freshwater runoff were >2.4 times more likely to die with significant protozoal disease. Interestingly, none of 138 POPs tested in the current study significantly increased the risk for otters dying with protozoal meningoencephalitis or systemic protozoal disease. In fact, otters with high liver levels of PCB 056 were significantly less likely to die with protozoal disease, as seen in the adjusted odds ratios (Table 17), with every 0.1 decrease in PCB burden increasing the risk of meningoencephalitis by approximately 3 times. Three factors associated with a decreased risk of sea otter death due to protozoal disease were low exposure to municipal wastewater effluent, otters stranding as adults and carcass recovery from the relatively pristine Big Sur coastline (1.9 times, 1.9 times, and 5.6 times less likely, respectively).

Table 17. Univariate model, showing associations between liver POP concentrations in sea otters and death due to systemic protozoal disease

	Chi 2 P-Value	Students T P-Value	Crude OR	95% Upper	95% Lower	Adjusted OR	95% Upper	95% Lower
Big Sur	0.010		0.177	0.773	0.04			
Adult	0.020		0.526	0.933	0.296			
High freshwater runoff exposure	0.029		2.466	5.638	1.078			
Low wastewater exposure	0.040		0.518	0.976	0.275			
Benzo(e)pyrene		0.035	N/A	N/A	N/A	N/A	N/A	N/A
BIPHENYL		0.041	0.463	1.035	0.207	0.530	1.177	0.239
PBDE_028		0.009	2.117	4.633	0.967	2.341	5.430	1.009
PCB_056		0.034	0.000	1.270	0.000	0.000	0.635	0.000

All infectious causes of mortality

Univariate associations for liver POPs and other risk factors in relation to death from all major infectious causes of disease that were either primary causes of death or one of the two main contributing causes of death are summarized in Table 18. Otters with scant body fat at necropsy had a 2.4 times higher risk of having an infectious disease as compared to all other nutritional groups. Crude odds ratios revealed an increased risk of death from infectious disease for otters with livers containing high concentrations of PCB 66, 74 and 105; however, these findings were not longer significant when adjusted for sea otter age, sex, location and nutritional condition. A decreased risk of death due to all causes of infectious disease was noted for adult otters, animals with moderate body fat and otters recovered along the Big Sur coast (approximately 3.2-3.7 times lower risk for each). Finally, otters stranding in coastal regions with low municipal wastewater exposure were >6 times less likely to die with significant infectious disease, compared to otters stranding in areas with moderate wastewater exposure; no otters were recovered from areas impacted by heavy wastewater outflows in the present study.

Table 18. Univariate model, showing associations between liver POP concentrations in sea otters and death due to infectious disease

	Chi 2 P-Value	Students T P-Value	Crude OR	95% Upper	95% Lower	Adjusted OR	95% Upper	95% Lower
Big Sur	0.010		0.322	0.781	0.133			
Adult	0.000		0.311	0.619	0.156			
Scant Fat	0.025		2.412	5.296	1.098			
Moderate Fat	0.000		0.269	0.522	0.139			
Low wastewater exposure	0.000		0.163	0.325	0.082			
DDD(p,p')		0.022	1.028	1.062	0.995	1.008	1.040	0.977
DDE(p,p')		0.016	1.000	1.001	1.000	1.000	1.000	0.999
trans-Nonachlor		0.045	1.007	1.025	0.989	0.993	1.010	0.976
Naphthalenes, C4 -		0.039	0.551	1.038	0.293	0.683	1.425	0.328
PBDE_047		0.033	1.006	1.017	0.994	0.996	1.006	0.986
PBDE_085		0.024	N/A	N/A	N/A	N/A	N/A	N/A
PBDE_100		0.037	1.024	1.062	0.986	0.996	1.031	0.962

	Chi 2 P-Value	Students T P-Value	Crude OR	95% Upper	95% Lower	Adjusted OR	95% Upper	95% Lower
SUMPBDE		0.032	1.003	1.009	0.998	0.999	1.003	0.633
PCB_052		0.033	1.224	1.555	0.964	1.044	1.205	0.903
PCB_066		0.003	2.154	3.794	1.223	1.527	2.824	0.826
PCB_074		0.008	2.492	5.343	1.163	1.441	3.244	0.640
PCB_087		0.026	1.140	1.333	0.976	1.035	1.146	0.936
PCB_095		0.030	1.162	1.387	0.973	1.035	1.154	0.928
PCB_097		0.042	1.134	1.401	0.918	1.030	1.149	0.924
PCB_099		0.007	1.057	1.125	0.993	1.011	1.048	0.976
PCB_101		0.025	1.043	1.097	0.992	1.010	1.041	0.980
PCB_105		0.005	1.158	1.329	1.008	1.028	1.133	0.933
PCB_110		0.016	1.178	1.405	0.987	1.062	1.221	0.924
PCB_118		0.005	1.026	1.062	0.991	1.002	1.021	0.985
PCB_128		0.011	1.101	1.227	0.988	1.020	1.089	0.956
PCB_137		0.036	1.276	1.809	0.900	1.032	1.238	0.859
PCB_138		0.017	1.011	1.028	0.994	1.001	1.010	0.993
PCB_141		0.033	1.307	1.808	0.945	1.066	1.336	0.851
PCB_151		0.046	1.120	1.279	0.981	1.024	1.117	0.938
PCB_153		0.042	1.005	1.015	0.995	1.000	1.006	0.995
PCB_156		0.036	1.085	1.262	0.933	1.012	1.095	0.935
PCB_158		0.018	1.207	1.535	0.948	1.038	1.183	0.910
PCB_183		0.035	1.107	1.245	0.984	1.017	1.105	0.936
PCB_200		0.035	1.804	3.330	0.977	1.183	1.924	0.728
PCB_209		0.039	1.801	3.337	0.972	1.259	2.030	0.781
SUMPCB008-101		0.013	1.015	1.033	0.999	1.003	1.013	0.993

Multivariate associations of POP test results with major findings at necropsy

Overview

The results for multivariate studies differ from those of the univariate analyses provided above in that they reflect the best fit of a model that is developed to support the observed data. Factors incorporated into multivariate models include those that are most likely to significantly impact the outcome in question; these impacts may be either positive or negative (e.g., risk factors associated with an increase or decrease in hepatic SumPCBs). Risk factors with a P value <0.05 and odds ratios >1 significantly enhance an animal's risk of having the condition in question, while odds ratios <1 decrease the overall risk (i.e., are "protective"). If the 95% CI includes 1, it is possible that the observed effect could have occurred through random chance.

Trauma

Multivariate associations for liver POP concentrations and other risk factors in relation to otter death due to trauma are summarized in Table 19. As with the univariate analyses, adult otters were twice as likely to strand with significant trauma than other age classes. Otters stranding along the coast north of Santa Cruz were nearly 5.5 times more likely to have died with significant trauma, especially trauma due to shark predation. In contrast to the univariate analyses, otters in good nutritional condition and those from Monterey Bay were not significant risk factors for traumatic death in otters.

Similar to the univariate models, only the presence of C1-dibenzothiophene in liver tissue was associated with a significantly increased risk of trauma; every 1 unit of increase in this compound increased an otter's risk of trauma by 3 times. To better characterize this risk for the observed range of dibenzothiophene in sea otter livers; each increase of 0.620 ng/g (2 SD) of C1-dibenzothiophene, increased an otter's risk of death with significant trauma by 2.74 times. Conversely, a minimal "protective" effect for death due to trauma was identified for otters with higher liver levels of dieldrin (Table 19). When adjusted for the range of dieldrin observed in sea otter livers, each increase of 22.4 ng/g (2 SD) decreased the risk of otter death due to trauma by 3.07 times, suggesting a "protective" effect for dieldrin.

Table 19. Multivariate model, showing associations between liver POP concentrations in sea otters and death with significant trauma

Risk Factor	Odds Ratio	95% CI
Adult	2.194	1.216-3.956
Dibenzothiophene, C1-	2.803	1.179-6.662
North Coast	5.484	1.290-23.312
Dieldrin	0.952	0.912-0.993

Bacterial disease

Multivariate associations for liver POPs and other risk factors in relation to death due to bacteria are summarized in Table 20. Similar to the univariate comparisons, otters stranding within Monterey Bay had an increased (>1.8 times) risk for death due to bacterial disease, and immature otters and those with moderate body fat were 3.5 to 5 times less likely to have primary bacterial infections at the time of stranding. Similarly, liver concentrations of PCB 056 remained highly correlated with death with bacterial infection; each 0.15 ng/g (2 SD) increase in PCB 056 increased an otter's risk of death with significant bacterial disease by 2.76 times. In contrast to the univariate models, aged adult otters, those with scant body fat, and those with high liver concentrations of PCB 66 and 70 did not have an enhanced risk of death due to bacterial disease in multivariate studies.

Table 20. Multivariate model, showing associations between liver POP concentrations in sea otters and death with significant bacterial disease

Risk Factor	Odds Ratio	95% CI
Moderate Fat	0.288	0.156-0.532
Immature	0.201	0.084-0.484
PCB 056	947.989	3.105-289,404.1
Monterey Bay	1.872	1.056-3.318

Acanthocephalan peritonitis

Multivariate associations for liver POPs and other risk factors in relation to death due to acanthocephalan peritonitis are summarized in Table 21. An increased risk of dying with moderate to severe acanthocephalan peritonitis was associated with otters having high liver concentrations of oxychlordane and cis-chlordane. When the odds ratios were adjusted for the actual range of oxychlordane observed in sea otters, each 11.7 ng/g (2 SD) increase in oxyclordane increased an otter's risk of death with moderate to severe acanthocephalan peritonitis by 59.85 times. Similarly, for every 2.9 ng/g (2 SD) increase in cis-chlordane concentration in otter liver, a 3.23 times increased risk of significant acanthocephalan peritonitis was noted. Interestingly, otters stranding in areas of low exposure to municipal wastewater were significantly less likely to die with significant acanthocephalan peritonitis in both the univariate and multivariate analyses. A protective effect was also noted for otters exposed to trans-nonachlor, with every 44.8 ng/g (2 SD) increase of transw-nonachlor in liver tissue resulting in a 36.76 times decrease in the risk of dying with moderate to severe acanthocephalan peritonitis.

Table 21. Multivariate model, showing associations between liver POP concentrations in sea otters and death with significant acanthocephalan peritonitis

Risk Factor	Odds Ratio	95% CI
Low wastewater exposure	0.063	0.015-0.272
Oxychlordane	1.416	1.182-1.696
trans-Nonachlor	0.923	0.881-0.967
cis-Chlordane	1.497	1.073-2.090

Protozoal disease or meningoencephalitis

Multivariate associations for liver POPs and other risk factors in relation to death due to systemic protozoal infection are summarized in Table 22. One factor that significantly reduced the risk of sea otters dying with systemic protozoal disease was carcass recovery from the Big Sur coastline (7.5 times less risk). Similar to previous studies from the same region (Miller et al., 2002b), otters stranding in areas exposed to heavy freshwater runoff were nearly 2.5 times more likely to die with significant protozoal disease. A similar level of increased risk was associated with otters testing strongly positive for PBDE 028, a tri-PBDE. Paradoxically, otters testing strongly positive for PCB 056 were significantly less likely to die with moderate to severe protozoal disease. When the odds ratios were adjusted for the actual PBDE 028 concentrations observed in sea otter livers, each 2 SD increase increased an otter's risk of dying with moderate to severe protozoal disease by 1.91 times. In contrast, each 2 SD increase in PCB 056 concentrations in liver decreased the risk of moderate to severe protozoal disease in otters by 4.35 times.

Table 22. Multivariate model, showing associations between liver POP concentrations in sea otters and death with significant systemic protozoal disease

Risk Factor	Odds Ratio	95% CI
Big Sur	0.134	0.025-0.713
Adult	0.519	0.282-0.956
PBDE 028	2.369	1.069-5.251
PCB 056	0	0.000-0.590
High freshwater runoff exposure	2.419	1.008-5.804

All infectious causes of mortality

Multivariate associations for liver POPs and other risk factors in relation to death due to all major infectious causes are summarized in Table 23. As was seen below, the final model had no POP contaminants that significantly improved the fit of the cumulative infectious disease model beyond that achieved by incorporating various demographic, spatial and environmental risk factors.

A significantly decreased risk of death due to all causes of infectious disease was observed for otters stranding along the remote and comparatively pristine Big Sur coast, and for otters dying in good nutritional condition. As with the univariate analyses, otters exposed to low municipal wastewater outflows were nearly 5 times less likely to die with significant infectious disease when compared to otters exposed to moderate wastewater outflows.

Table 23. Multivariate model, showing associations between liver POP concentrations in sea otters and death with significant infectious disease of all causes

Risk Factor	Odds Ratio	95% CI
Low wastewater exposure	0.204	0.099-0.420
Moderate Fat	0.27	0.127-0.57
Big Sur	0.232	0.083-0.644

DISCUSSION

Overview

Here we present the results of a 5 year study focused on examining associations between liver concentrations of persistent organic pollutants (POPs) and various demographic, spatial and environmental risk factors, as well as major causes of death in threatened southern sea otters (*Enhydra lutris nereis*). Samples from 227 freshly dead sea otters stranding between 2000 and 2005 along the California coast were included; these samples were tested for the presence of most major classes of POPs, including PCBs, PBDEs, PAHs, organochlorine pesticides and organotins. In addition to the top 3 causes of death, other potential contributors to the risk of POP exposure that were also considered in the various statistical models included sea otter sex, age class, nutritional condition and stranding location, as well as the proximity of each stranding location to major points of coastal freshwater runoff, municipal wastewater discharge and areas of dense coastal human populations. What follows is a discussion of the major findings of this research, organized by each major topic, including discussion of major risk factors for POP exposure in sea otters. Because some of the conclusions are preliminary and additional analyses are in progress, caution is advised in interpretation of these findings, particularly with respect to liver POP concentrations in sea otters and major causes of sea otter death.

Sex

In the current study, adult female otters had significantly lower liver concentrations of many POPs. One major factor causing differences in liver POP concentrations between males and females is progressive mobilization of body POP stores as a result of pregnancy and lactation in adult females (Wolkers, 2006). Conversely, since male sea otters accumulate many POPs throughout their lifetimes, studies of POP levels utilizing small, mixed gender samples with a predominance of males may overestimate population-level contaminant burdens, as well as potential contributory affects of these pollutants to disease. Most POPs analyzed for this project are hydrophobic, lipid-soluble chemicals that are sequestered into fat after ingestion. Many of the examined POPs have the potential to bioaccumulate in adipose tissue. In contrast to all other species of marine mammals, sea otters have very limited fat stores that can be rapidly mobilized and metabolized when the animals are not foraging adequately or during pregnancy and lactation. This ability to reduce tissue POP burdens through gestational and lactational transfer may explain the higher concentrations of DDT metabolites, PBDEs, and tetrachlorinated PCBs detected in adult male otters, when compared to females.

Differential mobilization of various POPs may also be occurring: In the current study, hexa-, octa-, and nona-chlorinated PCBs were 1.5-2.9 times more concentrated in livers of females than in males. Conversely, tetra-chlorinated PCBs were 1.7-2.8 times more concentrated in livers of males than in females. This pattern of higher chlorinated compounds being more concentrated in females and lighter, tetra-chlorinated compounds in being higher in livers of male otters could be because lighter, tetra-chlorinated compounds are more readily mobilized during gestation and lactation than heavier, more densely chlorinated PCB congeners. This hypothesis will be considered for future studies; it may be possible to address this question by examining the hepatic concentrations and ratios of PCB homolog groups in sea otter pups and comparing them with those from adults of both sexes.

Age

Two opposing patterns of POP concentrations by age class were detected in the present study: 1) aged adults had significantly higher concentrations of organotins and acenaphthylene than

immatures, and 2) immatures had significantly higher concentrations of pesticides, PBDEs, and PCBs than adults. These differences may be due to variations in maternal transfer of POPs, metabolism, growth and foraging-related dilution of the initial dose of tissue POPs obtained during gestation and nursing and/or postweaning bioaccumulation. In the current study, immature otters had significantly higher liver concentrations of pesticides, PBDEs, and PCBs than did adults, likely due to mobilization of lipid-soluble POPs from the maternal fat reserves during lactation. Adult female otters can reduce their total body of POPs in this manner, but this route ensures that her small, rapidly growing pup is fed fat-rich, and likely POP-contaminated milk. With each new pup produced, an adult female may be able to sequentially reduce her tissue burden of fat-soluble POPs, in contrast to the males. Conversely, females have high metabolic demands during pregnancy and lactation and may replenish or augment their tissue POP burdens as a result of increased foraging activity during this time period. It is likely that all POPs are not equally mobilized from fat. Thus liver POP concentrations in adult females may reflect both bioaccumulation of POPs from enhanced foraging activity and the spectrum of POPs that are most easily mobilized from body fat stores. Another possible explanation is that after weaning, tissue POP burdens do not increase proportionately with increases in body mass, leading to a dilutional effect. In the current study, immature sea otter POP concentrations were significantly higher than those of adults for several POPs. In addition to maternal transfer, this pattern could also be due in part to this dilutional effect, and would explain why hepatic levels of POPs in younger male otters are lower than those in adult males. There was also a small increase in POP concentrations for aged adults that could result from progressive bioaccumulation, declines in nutritional condition with mobilization of body fat stores or a reduced ability to metabolize these contaminants.

Aged adults had significantly higher concentrations of organotins and acenaphthylene than immatures. Organotins and acenaphthylene are not lipid soluble and are not sequestered into fat, therefore these compounds are less likely to be mobilized during lactation and are therefore less likely to be transferred from the mother to the pup. Without significant lactational transfer of organotins or acenaphthylene, the pattern observed is more comparable to the classic model of bioaccumulation of contaminants with increasing age, instead of starting with high initial doses as pups as a result of maternal transfer.

Nutritional Condition

In this study, POP concentrations were measured in liver tissue from freshly dead sea otters. Due to sea otters' limited fat stores and their high resting metabolic rate, emaciated sea otters are likely to die within days of mobilizing the last of their energy stores if they are not able to forage adequately. Our results show that emaciated animals had the highest liver levels of contaminants and that liver concentrations of most POPs vary inversely with nutritional condition. Because of this, nutritional stress may result in marked, and often rapid transport of DDT and other fat-soluble contaminants from adipose tissue to the liver. This pattern of POP mobilization in relation to body condition may be enhanced by pregnancy and lactation, as described above, as well as by illness. Emaciated animals with no subcutaneous fat stores had the highest liver concentrations of POPs, while animals with abundant subcutaneous fat had the lowest concentrations. As body fat is mobilized, fat-soluble compounds such as DDT and its metabolites may be mobilized to the liver for detoxification. The rapid weight losses reported for nutritionally stressed sea otters may result in a very high and rapid pulse of POP loading of metabolically active hepatocytes, at a time when animals are least capable of enduring the ill effects. In prior studies, high POP exposures have been causally linked with weight loss in exposed humans and laboratory animals (Carter and

Preston, 1983; Bowers , 2003; Siddiqi et al. 2003). Thus in emaciated sea otters it is possible that these negative effects could result in rapid accentuation of an existing negative energy balance, causing a negative feedback loop and leading to enhanced, POP-associated morbidity and mortality. These impacts would be difficult to detect in opportunistic analysis of stranded animals, as in the present study, but these observations may be critically important for this threatened species. For example, in the southern sea otter population, one major bottleneck regarding sea otter recovery appears to be heavy losses of reproductively active adult females that are approaching the end of lactation; this timepoint appears to be the most energetically demanding part of the sea otter life cycle. These end-lactation females animals often present for necropsy with severe emaciation. If the negative feedback loop described above exists for sea otters, this critical component of the southern sea otter population would be expected to be the most vulnerable. A second, highly sensitive group would be the recently weaned, immature otters of both sexes that are just learning to forage independently.

Region

Pesticides

In the present study, higher concentrations of POP pesticides were detected in livers of sea otters that stranded in Monterey Bay, when compared to other areas. This finding is likely to be associated with the drainage of agricultural soils into Monterey Bay. The Pajaro and Salinas river valleys have significant acreage devoted to irrigated agriculture; these fields have been treated with various pesticides, including DDT, for decades (Mischke, 1985). During the rainy part of each year, pesticide-contaminated soils are washed into Monterey Bay via the Salinas and Pajaro Rivers, among others (Paull, 2002) (Figure 17). The National Status and Trends Mussel Watch Project have had monitoring stations located at the opening of Elkhorn Slough in the Moss Landing Harbor, as well as outside of the harbor at the Moss Landing Pier since the early 1990s. . The average sumDDT concentrations in mussels collected from Elkhorn Slough for all years rank third highest out of 39 NST Mussel Watch sites statewide (Figure 18).

Figure 17. Map of Monterey Bay showing the location of the Salinas and Pajaro valleys, Moss Landing, Santa Cruz and Monterey with respect to the upper and lower Monterey submarine Canyon. Red arrows indicate sediment transport directions through the agricultural valleys. White arrows indicate the dominant longshore currents. Orange arrows indicate inferred sediment transport direction addressed in this paper (Figure reprinted from Paull, 2002).

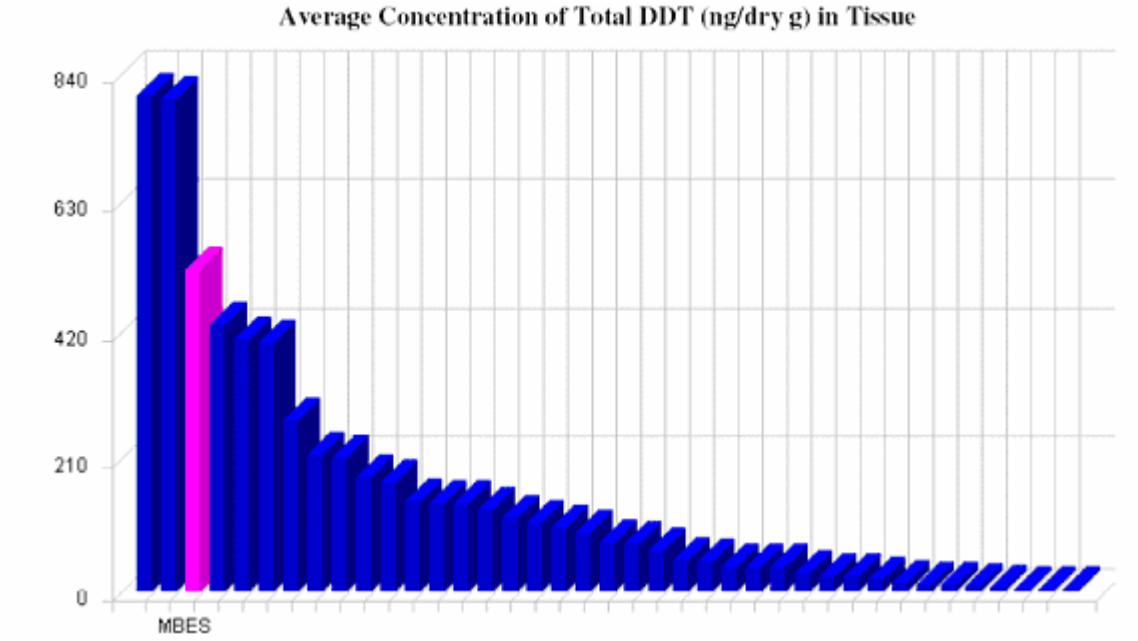

Figure 18. The National Status and Trends Mussel Watch site at Monterey Bay, Elkhorn Slough (MBES) ranks 3rd out of 39 sites monitored for sumDDT in California. The average concentration of sumDDT in mussels collected at MBES is 525.06 (ng/dry g). Only the San Pedro and Palos Verdes drainages in southern California have higher average sumDDT concentrations in mussels of 39 sites monitored throughout California.

PAHs

High concentrations of acenaphthylene were detected in livers of sea otters from the south coast region, when compared to the north coast. This finding may be explained by the presence of numerous natural seeps along the coast of southern California. Dugan et al., (2005) analyzed sand crabs from several beaches within the sea otter range, from Scott Creek to the north, and Carpenteria Marsh to the south; she found the highest mean PAH concentrations in sand crabs were from those collected near the Santa Maria River. At this site, the Guadalupe oil field may be a source of elevated PAH levels found locally in sand crabs and sea otters. However, natural seeps are also a source of PAHs and these natural phenomena are not uncommon along the coast of southern California. Only 33 of the other 46 PAHs tested in the present study were detected in southern sea otter livers, possibly due to their rapid metabolic breakdown in the liver post-ingestion (Kahn, 1989).

PCBs

In the current study, otters from Monterey Bay exhibited elevated PCB levels for half of the congeners significantly affected by region, when compared to Estero Bay animals. This finding could be related to the continued use of PCBs as carrier compounds for pesticides, called pesticide extenders (Agency for Toxic Substances and Disease Registry, 2000).

Exposure to freshwater runoff, wastewater discharges and proximity to dense human populations

Freshwater runoff can convey contaminants to nearshore waters from various sources. Three of the larger watershed in the study area, the Pajaro, Salinas and Santa Maria rivers include large areas of irrigated cropland that have historically received treatments with pesticides. These pesticides have included legacy chlorinated compounds, such as DDTs and HCHs. DDT degrades in the environment to form DDE and DDD; higher concentrations of DDE and DDD homologues in otters stranding near moderate sources of freshwater runoff, compared to low sources of runoff, is consistent with the legacy agricultural use and slow environmental degradation of DDT. Some coastal watersheds, such as the San Lorenzo, Pajaro, Salinas and Carmel rivers, can also convey runoff from urban and suburban sources; higher concentrations of the combustion product dibenzothiophene-C2 and PBDE 017 in sea otters with moderate or high exposure to freshwater runoff are consistent with urban sources. Dibenzothiophene-C2 is likely being deposited on streets from vehicle exhaust, from where it is then washed into watersheds and, ultimately, the ocean via precipitation. PBDEs have various origins, being included in furniture and electronics as flame retardants. The general absence of significantly higher concentrations of POPs in otters with high exposure to freshwater runoff is likely the result of the low number of samples available from otters stranding near areas of high freshwater runoff.

Otters with higher exposures to wastewater also had higher concentrations of one DDE homologue and the combustion product dibenzothiophene-C2, similar to otters with greater exposure to freshwater runoff. These two compounds suggest contamination from the same sources, as described above. Significantly higher concentrations of four PBDE congeners in sea otters that stranded in areas with moderate exposure to wastewater discharges are consistent with previous reports of high concentrations of these contaminants in wastewater (Anderson and MacRae, 2005; Ikonomou and Rayne, 2005). Higher concentrations of PCB 200 and PCB 209 in sea otters with low exposure to wastewater is enigmatic, although they suggest that wastewater is not a source for these contaminants.

Of the three POPs and total organotins that varied significantly in sea otter livers according to human population density, dibutyltin, the sum of organotins and PBDE 183 suggested a positive association between increased human population densities and higher liver concentrations of these compounds in sea otter stranding nearby. PBDE 183 was higher in the two highest categories of human population density, although otters stranding in areas with human population between 3001-6000/mile^2 had higher concentrations than for those stranding in areas with higher population density. This likely reflects the fact that comparatively few otters were recovered offshore of the most densely settled coastal regions. Organotins are associated with application of antifouling paint on boats and would be expected to be highest in otters residing near harbors, which are not always situated in areas with dense human populations. For dibutyltin and sum of organotins, the highest liver concentrations were detected in sea otters stranding in areas with the highest human population density, although there was no significant difference between otters from locations with the lowest and highest human population densities. Again, this lack of difference is likely due in part to low numbers of samples in the category of high human population density, as well as high within-category variation with respect to liver POP concentrations. The PAH acenaphthylene had highest concentrations in otters stranding in areas with the lowest human population, although it was not significantly different from the two highest categories of human population density. Acenaphthylene is a three-ring PAH that is more

characteristic of petrogenic PAHs than of pyrogenic PAHs, and may be associated with natural seepage along the central California coast.

Associations of POP concentrations between sea otters and mussels

Mussel POP concentration was a significant covariate affecting sea otter POP concentration for the following POPs: sumDDT, sumChlordanes, sumPCBs, and dieldrin. This covariate was significant in the model including the categorical factors of age, gender, and body condition. These results suggest that the POP concentrations in filter-feeding mussels are statistically and positively correlated with the POP concentrations in sea otter livers for sumDDT, sumChlordanes, sumPCBs, and dieldrin.

Mussels may account for 0-47% of the wet edible biomass of a sea otter diet (Tinker et al., 2007) and are stationary filter-feeding bioindicators that provide an approximate measure of the bioavailable POP contaminant load in filter-feeders for a particular area. Our results suggest that mussel concentrations at the five NST sites used in this study may have predictive value for monitoring the POP burdens for sea otters stranding nearby. However, demographic factors of age, sex, and body condition must also be considered in trying to determine the key risk factors for development of high liver POP burdens.

Significant interactions were detected between sea otter gender, sumDDT concentrations in mussel tissues and sumDDT concentrations in livers of sea otters. In particular, strong positive correlations were detected for liver vs. mussel sumDDT concentrations for female otters, but not for males. This may be due to differences in home range size between males and females (Ralls et al., 1996). Because of their smaller home ranges, female sea otters' sumDDT concentrations may be better reflected by mussel sumDDT concentrations for a given area than the matching values for male otters.

Associations with disease

With respect to the primary and contributing causes of otter death, associations with liver POP concentrations were less clear-cut than in previous sea otter studies where sample sizes were smaller and case selection and statistical evaluation less rigorous than in the present study. In prior studies, otter sample sizes ranged from 8 to 80 (Table 24) and stratification of the various risk groups ranged from none (Bacon et al., 1999), to case selection by specific coastal location with stratification by categorical cause of death (Nakata et al., 1998) to more rigorous stratification (Kannan et al., 1998). In some studies, animals diagnosed with emaciation as a cause of death were included as a single risk group, without adjusting results from other animals for variations in nutritional condition (Kannan et al., 2006a; 2006b). This approach may lead to erroneous conclusions, because data from the current and prior studies demonstrate significant inverse correlations between the nutritional condition of stranded sea otters and other marine mammals and elevated liver concentrations of most POPs (Table 7). Nutritional condition is also inversely correlated with some common infectious and traumatic causes of sea otter death, such as bacterial infection and mating trauma, but not with others, such as acute death due to shark bite or boat strike (Kreuder et al., 2003). Thus consideration of nutritional condition for all animals is imperative for studies on effects of POP exposure in sea otters. Further, case selection in some prior studies was based on very broad criteria for identification of adult otters, thus allowing for inclusion of subadult and immature animals in treatment groups defined as "adults" (Kannan et al., 1998; Kannan et al., 1999; Kannan et al., 2004). As a result of the age class-based data stratification used in the current study, a progressive, age-related decline in concentration of some

liver POPs was noted (Figures 6, 8, and 9), while for others, liver concentrations increased progressively with age (Figure 7). Thus, inadvertent or intentional inclusion of young or aged otters in an "adult" age group could significantly skew the results of POP analyses, leading to erroneous conclusions.

In the present study, when multivariate logistic regression models were used to adjust for age, sex, nutritional condition, sample location and other factors, no significant positive or negative disease correlations were found for summed butyltins, PBDEs, PCBs, DDTs and all other pollutants examined, when examined with respect to the top 3 findings at necropsy. However, liver concentrations of selected POP analytes were significantly correlated with the presence of specific infectious diseases, as well as traumatic death (Tables 13, 14, 15 and 16). Otters having high concentrations of dibenzothiophene were more likely to die with significant trauma. Elevated liver levels of PCB 056 were associated with death due to bacterial infection, but were inversely correlated with death due to systemic protozoal infection. In contrast, otters testing strongly positive for PBDE 028 were more likely to die from protozoal disease. An increased risk of death due to acanthocephalan peritonitis was found for otters testing strongly positive for oxychlordane and cis-chlordane. However, when all infectious causes were pooled into a comprehensive multivariate model, no POP analytes were found to positively correlate with sea otter death due to infectious disease, when the top 3 causes of death were considered.

In the present study, otters testing strongly positive for coplanar PCB 056 were significantly less likely to die with moderate to severe protozoal disease, but had an increased risk of death due to bacterial disease. This interaction is most likely explained from a statistical perspective and not a biological association. Because the study group is a closed group and no true "controls" could be included, the most likely explanation for the "protective" nature of PCB 056 towards protozoal disease is that animals with bacterial infection are likely to die from that cause without developing concurrent protozoal encephalitis. Thus, an increased incidence of bacterial disease would be directly responsible for the decrease in protozoal encephalitis, therefore the increased concentrations of a contaminant would likely be reflected in the subsequent decreases in those animals without the disease. An alternative hypothesis is that PCB 056 mediates immune responses to both bacterial and protozoal infections, and in sea otters these effects may enhance risks for illness and death for some pathogens, while reducing them for others.

Potential metabolic and cytochemical mechanisms of POP toxicity

Little information is currently available on the specific toxic effects and cellular mechanisms of toxicity of PCB 056. In marine mammals, potential toxic effects of PCBs reported in prior studies include endocrine disruption (Simms et al., 2000; Troisi and Mason, 2000), reproductive impairment (DeLong et al., 1973) and cancer (De Guise et al., 1994a; 1994b). Also both *in vivo* and *in vitro* studies suggest that organochlorines can be immunotoxic in marine mammals (Levin et al., 2007). In one recent study, both coplanar and non-coplanar PCBs affected the respiratory burst of leukocytes isolated from mice, humans, cetaceans and pinnipeds. However, the specific effects on different leukocyte sub-populations varied and both additive and inhibitory effects were identified, suggesting that the cytochemical pathways modulating these effects also varied by animal species and PCB analyte (Levin et al., 2007). An enhanced risk of developing disease could accompany both up- and downregulation of the respiratory burst or other immune mechanisms during the normal host response to bacteria, parasites and fungi: Depletion or decreased production of respiratory burst-mediated reactive oxygen species could render the host less able to effectively kill pathogens, when encountered. Conversely, enhanced production of

reactive oxygen species could lead to excessive host tissue damage (Levin et al., 2007). For PCB-exposed southern sea otters, an enhanced risk of death to infectious agents could result from both ineffective mechanisms for pathogen inactivation or an exaggerated host immune response. There is histological evidence to suggest that the latter may occur in some systemic protozoal infections (Miller, unpub. data).

In addition to the innate immune impairments described above, both coplanar and nonplanar PCBs have been associated with suppression and upregulation of humoral immunity in adult and neonatal goats (Lyche et al., 2006). Potential cell-mediated effects of PCB exposure have not been systematically evaluated, in humans, laboratory animals or wildlife, in part due to the expense and technical difficulty of doing so. Thus, although it is tempting to speculate that differences in the risk of mortality due to protozoa and bacteria in relation to PCB 056 exposure could be mediated by innate, humoral or cell-mediated immune impairments, independent confirmation of these preliminary findings and careful study are needed.

Sea otters with higher liver concentrations of PBDE 028 were more likely to die with significant protozoal disease (Table 17). Some specific mechanisms of PBDE toxicity that have been proposed for humans and other mammals include induction of metabolic enzymes (e.g., cytochromes), suppression of enzymes that serve as protective scavengers of free radicals (e.g., GSH reductase) and thyroid disruption through TSH suppression and decreased thyroxine secretion, (Darnerud, 2003). Other studies have suggested a role for PBDEs in the aryl hydrocarbon receptor (AhR) signal transduction pathway as agonists, antagonists or both (Sanderson et al., 1996). The AhR mediates many of the biological and toxicological actions of dioxin and related pollutants. Impaired motor function, habituation, learning and memory have been documented in mice exposed to penta-PBDEs as fetuses or neonates (Eriksson et al., 2001; 2002a; 2002b; Branchi et al., 2002). Little specific information has been reported for immunotoxic properties of PBDE 028. Since host immunity toward intracytoplasmic protozoa such as *T. gondii* and *S. neurona* is primarily mediated through cytokine and cell-mediated pathways (Tenter et al., 2000), a specific mechanism for PBDE effect on these pathways should be considered, particularly for cells of the central nervous system and for inflammatory cells such as macrophages and lymphocytes. Reported neuronal effects of lower-brominated PBDEs, such as PBDE 028 are induction of phospholipase A_2 in cerebellar granule cells, possibly leading to cell death, and decreases in conduction velocities for sensory and motor neurons (Darnerud, 2003). Neurological disease is commonly reported from live-stranded sea otters (Miller, 2007 in press); in most cases, these deficits are attributed to hypoglycemia, hyperthermia, hypothermia, protozoal disease or biotoxin exposure. However, given the high concentrations of PBDEs observed in marine mammals, including sea otters, in this and prior studies (She et al., 2002), the possibility that PBDEs could increase the risk of sea otter death indirectly through negative effects on behavior, cognition and foraging activity should be investigated. Additional analytical approaches will be needed to fully explore these various hypotheses.

Are POPs associated with a decreased risk of sea otter death?

Surprisingly, high liver levels of some POPs were associated with a lower risk of otter death due to specific infectious diseases: otters testing high positive for dieldrin were slightly less likely to have trauma as a significant cause of death. A slight "protective" effect against development of acanthocephalan peritonitis was also noted for otters with high liver levels of trans-nonachlor, and otters testing strongly positive for PCB 056 were less likely to die from systemic protozoal disease. As was described above for PCB 056, these "protective" contaminant burdens are likely a

statistical artifact of how the different disease processes interact with one another versus a true "protective" effect resulting from increased body burdens of a given contaminant.

Contributions of risk due to spatial, demographic and environmental risk factors

This study also identified strong associations between sea otter death due to specific infectious diseases and a range of demographic and environmental risk factors, such as sea otter age, sex and nutritional condition, as well as each animal's proximity to major coastal discharges of freshwater runoff or municipal wastewater. In several cases, the risk of disease associated with these environmental factors was equal to or higher than the risk attributed to elevated liver POP levels. For example, adult otters were twice as likely to strand with trauma than other age classes, and otters stranding along the north coast were >5 times more likely to have died with significant trauma. This latter finding is not unexpected: A high frequency of shark attacks on sea otters have been reported from this same region in previous studies (Ames et al., 1996; Kreuder et al., 2003); at present, white shark attacks appear to be one key factor limiting northward range expansion for southern sea otters in California.

This is the first report confirming a higher risk of adult otter death due to trauma; this is partly because mating-related trauma, such as nose wounds, were included under traumatic causes of sea otter death in the present study. Male sea otters inflict these wounds by grasping the nose of the female with their teeth during mating (Reidman and Estes, 1990). Nose wounds may be observed on otters of either sex, and are sometimes inflicted on immature and subadult females, presumably due to forced mating. However, the vast majority of fresh nose wounds occur on adult and aged adult otters, especially sexually receptive females. In some cases these lesions are severe enough to cause death (Staedler and Reidman, 1993).

In the univariate models, otters dying with trauma tended to have more subcutaneous body fat, which is not surprising, given that traumatic death is often acute and these results are being compared to all otters in the study – many of which have subacute to chronic, debilitating diseases. This finding was not included in the final multivariate model, possibly because the North Coast and Adult risk groups explained much of this association (and fit better with the data), and also because some traumatic lesions, such as nose wounds are more commonly associated with moderate to severe emaciation. In the current study, both nose wounds and other, more acute causes of trauma such as such boat strike and gunshot were pooled for analysis. The association of traumatic deaths with North Coast otters also likely explains univariate findings for Monterey Bay otters were not significant in the final multivariate models.

In univariate comparisons, otters dying with moderate to severe bacterial infections tended to be thinner, older (i.e., aged adults) and tended to strand within Monterey Bay (Table 15). These general trends persisted in the final multivariate model. Associations between nutritional condition and otter death due to bacterial infection are not surprising: Animals dying due to primary or opportunistic bacterial infections often survive long enough to become cachexic prior to death. Because of their high metabolic requirements, otters lose weight quickly when foraging ceases due to illness. Also, old sea otters often have severe dental disease, including worn or fractured teeth with open pulp canals that provide portals of entry for bacteria. Finally, older, subdominant otters may sometimes be forced to forage in less optimal habitat and prey heavily on easy-to-catch crustaceans, such as sand crabs (*Emerita analoga*) that serve as intermediate hosts for pathogenic acanthocephalans. In addition to cachexia resulting from superinfection by

acanthocephalans, affected otters commonly develop secondary peritoneal or systemic bacterial infections.

The determination that Monterey Bay is a high risk area for otter death due to bacterial infection is a new finding that may be important. Unlike some other regions of the sea otter range, the Monterey Bay area is heavily urbanized and has a large number of nutrient-enriched rivers, streams, culverts and wastewater treatment plants that empty into a relatively small and contained area. Several rivers in this region, including the Salinas and the Pajaro, are known to be heavily contaminated agricultural drainages (Hunt et al., 2003, Anderson et al., 2003). Also, because the geography and orientation of this large embayment, it is conceivable that currents and winds could periodically entrap discharged river water and/or wastewater along the immediate shoreline, where sea otters could be heavily exposed.

In the present study, no clear associations were detected between sea otters with significant bacterial disease at death and prior exposure to freshwater runoff or wastewater. However, other recent studies of enteric bacterial infection in stranded southern sea otters have identified some associations (Miller et al., unpub. data). In the present study, all primary and contributing causes of bacterial infection were pooled and examined collectively. By comparison, a study that identified associations between enteric bacterial infections in otters and proximity to freshwater flow and wastewater effluent discharge at stranding was based on systematic isolation of opportunistic enteric bacterial pathogens from feces of live and freshly dead, stranded sea otters, and was not based on the primary or contributing causes of otter death (Miller et al., unpub. data).

Otters stranding in areas of low exposure to municipal wastewater were significantly less likely to die due to acanthocephalan peritonitis in both the univariate and multivariate analyses. This finding has not been reported in prior studies. However, comparatively few otters in our sample population were exposed to moderate levels of municipal wastewater, and none stranded near coastal areas that were heavily influenced by municipal wastewater. As a result, the significant associations detected between municipal wastewater exposure and death due to acanthocephalan peritonitis are based on comparatively few data points. This association could be related to biological factors that bias spatial distributions of the crustacean intermediate hosts or avian definitive hosts, rather than resulting from a direct cause and effect relationship of wastewater exposure on sea otter deaths due to acanthocephalan peritonitis. For example, many of the municipal wastewater outfalls examined in this study are located off sandy shorelines, which provide optimal habitat for sand crab intermediate hosts of pathogenic acanthocephalans. Nonetheless, this preliminary finding is intriguing and merits further study.

As with prior studies (Miller et al., 2002b), factors that significantly increased the risk of sea otters dying with systemic protozoal disease included younger age class and those stranding near areas of heavy coastal freshwater runoff. Two factors that reduced the risk of sea otter death due to significant protozoal disease were adult age class at stranding and carcass recovery from the Big Sur coastline, which is also consistent with prior studies (Miller et al., 2002b; Kreuder et al., 2003). In the univariate analyses, sea otters stranding near areas with low exposure to municipal wastewater were approximately half as likely to have died due to moderate to severe protozoal disease. Although it is tempting to assign importance to this observation relative to the deposition of cat feces into municipal wastewater systems, it is imperative to note that this finding was not significant in the final multivariate model. In addition, all causes of protozoal disease were pooled for analyses, and at least half were caused by *Sarcocystis neurona*, a parasite shed by wild

opossums that is unlikely to end up in municipal wastewater in significant concentrations. Univariate associations between low wastewater exposure and increased risk of death due to systemic protozoal disease could also be explained by indirect associations; for example if areas of municipal wastewater discharge are more likely to be located in rural areas near wetlands that provide optimal habitat for opossums and/or outdoor cats, spatial correlations with wastewater discharge could easily be detected, but would not be causally linked.

Relating the study findings to major categories of sea otter death

When the disease categories were selected for this study, one key objective was to group the data by major infectious causes of sea otter mortality (e.g., bacterial infection, acanthocephalan peritonitis and protozoal infection), while providing a relatively "clean" outgroup (e.g., trauma without concurrent infectious disease) for comparison of liver POP burdens. Testing a large sample of otters allowed us to stratify the various otter risk groups by gender, age class, nutritional condition and other significant factors that could falsely contribute to the perceived risk of POP exposure, while still permitting adequate sample sizes for statistical analyses relative to each categorical cause of death.

A second objective was to separate the major infectious causes of otter death by the dominant type of host immunological response required to fight infection, so that any significant POP associations could also be examined from a functional perspective. For the most common bacterial diseases affecting sea otters, the main components required for host defense would include humoral factors (e.g., antibodies) and phagocytic cells (e.g., neutrophils). In contrast, for sea otters infected with pathogenic intracellular protozoans such as *T. gondii* and *S. neurona*, the main host response would be mediated by mononuclear cells (e.g., lymphocytes, plasma cells and macrophages) and specific cytokines, such as gamma interferon (Tenter et al., 2000). For otters with acanthocephalan peritonitis, both humoral and cell-mediated host defenses could be elicited, because many otters with acanthocephalan infections also develop secondary bacterial disease (Kreuder et al., 2003). Thus, this latter group of otters could be expected to share common traits with both previous groups. Assuming that POPs cause an increased, and analyte-specific risk of otter death due to perturbations in the host immune response, we expected to find clear-cut differences in the range of POPs associated with otters dying with bacterial disease, when compared to systemic protozoal disease. Because animals with severe acanthocephalan peritonitis also commonly develop bacterial infections, these animals could be expected to share some high-risk analytes with both groups. In practice, our results showed some hints of variation along cell-mediated and humoral immune response pathways, especially for PCB 056. However, due to the marked complexity of POP interactions with metabolic, cytochemical and subcellular processes in living systems, no definitive conclusions can be made at this time and additional study is needed.

When all causes of infectious disease were pooled for univariate analysis, a ~75% lower risk of death due to infectious disease was noted for adult otters, when compared to other age classes, and for otters stranding along the Big Sur coast. In addition, otters stranding in areas impacted by municipal wastewater outflows were >75% more likely to die due to pooled causes of infectious disease, when compared to otters stranding near areas exposed to lower wastewater discharges. Importantly, these effects persisted in the final multivariate model. Taken together with concurrent studies that suggest an increased risk of otters being infected with opportunistic enteric bacterial pathogens, when exposed to wastewater effluent or runoff (Miller et al., unpub. data), these findings suggest that sea otter recovery could be significantly impacted by nutrient and pathogen-rich coastal discharges of any type. This could occur through direct or indirect

mechanisms: direct exposure to wastewater could result in pathogen exposure, while indirect effects of wastewater discharge could result in increased exposure to toxic diatoms that thrive in nutrient-enriched coastal waters, although rivers have been shown to be a larger source of nutrients than wastewater discharges. Studies to investigate these important associations are needed.

Comparison with prior studies

Several investigators have reported POP concentrations in sea otters along the west coast of North America, providing an opportunity to examine whether there have been temporal trends in POP exposure in otters. Shaw (1971) analyzed DDTs in 10 sea otters that died in the Monterey Bay area in 1969 and 1970. The concentration of total DDT residue in his samples averaged 2,888 ng/g. Nakata et al., (1998) analyzed 20 liver samples collected from 1988-1992 and found higher mean concentrations of PCBs, DDTs and chlordanes than those measured in the current study (Table 24). Nakata's DDTs concentrations averaged 1802 ng/g, below Shaw (1971) but above the current study. Bacon et al., (1999) reported concentrations of PCBs and DDTs in seven sea otter liver samples collected between 1988 and 1991. Their DDT concentrations also were intermediate between those reported by Shaw (1971) and the current study. When replicate values are considered for the entire sample populations from Shaw (1971), Nakata et al., (1998) and the current study (Figure 20), analysis of variance reveals that the mean DDT concentrations from Shaw and Nakata did not differ significantly, but both were significantly higher than the present study ($r^2 = 0.125$, $p = >0.0001$). This decline is similar to that reported for DDT in California sea lions (Le Boeuf et al., 2002). The exception to this trend of declining POPs was dieldrin, which had higher mean concentrations in the current study than those reported by Bacon et al., (1999).

Five prior studies also examined associations between tissue POP concentrations and categorical causes of death in southern sea otters (Table 24). The first was based on analyses of total organochlorines and PCBs in tissues from 20 animals that stranded in 1970 (Nakata et al., 1998). They found that otters dying due to infectious disease and miscellaneous causes had higher tissue concentrations of PCBs and DDTs than those dying due to trauma. However, the ranges in tissue concentrations varied widely between animals, and these findings were not statistically significant for total PCBs. DDT concentrations were significantly higher for otters dying due to infectious disease, versus those dying from trauma or unknown cause. Importantly, these data were not stratified for nutritional condition or gender, and both subadult and non-aged animals were included under the pooled category of "adults" for analysis (Table 24). In a second study, tissues from 35 California otters stranding between 1992 and 1996 were examined for concentrations of summed butyltins, as well as mono-, di- and tributyltins (Kannan et al., 1998) (Table 24). They concluded that sea otters dying from infectious disease had significantly greater butyltin concentrations in their tissues than those dying from trauma & other causes. Similar to the previous study, these data were not stratified by nutritional condition or gender, although emaciated otters were used as a comparison group, and roughly equal numbers of males and females were included. Similar to Nakata et al., 1998, the broad criteria established for identification of adult otters allowed for inclusion of younger age class, non-reproductive animals in the study. Finally, case selection for this study was based on each otter's proximity to potential high- and low-risk areas for butyltin exposure (i.e., the cases were not randomly selected), so the data are not necessarily reflective of TBT-based impacts to the broader southern sea otter population. Three additional studies (Kannan et al., 2006a; 2006b) were performed on the same tissues from 80 adult female southern sea otters stranding between 1992 and 2002. In the first, associations between liver concentrations of the perflorinated polymers PFOS and PFOA and the

categorical cause of death was examined, and significant associations were detected for otters dying due to infectious disease. In the second study, liver concentrations of 20 trace elements, including mercury, cadmium and copper were examined with respect to the categorical cause of death and concentrations of manganese, cobalt, zinc and cadmium were found to be elevated in diseased and emaciated sea otters relative to the "other" comparison group. In the third, PBDE and PCB concentrations were tested with univariate procedures for temporal trends and differences among regions and causes of death (i.e., infectious disease, other noninfectious causes, emaciation). Marginally significant declines were reported for PCBs over the period 1992-2002, PBDEs and PCBs were higher in emaciated otters, and PCBs were higher in otters stranding south of Cayucos than those stranding between Seaside and Cayucos. PBDEs or PCBs did not vary according to cause of death. These last three studies showed significant improvement in efforts at achieving statistically relevant sample sizes and reducing the effects of sex and age class, and the last study stratified data by location and nutritional status (i.e., emaciated animals with death attributed to starvation were pooled as one comparison group). Overall, these data provide interesting insights for comparison with the present study, but any direct comparisons must be made with caution due to the differing statistical techniques used. The current study has emphasized the importance of including demographic and environmental risk factors in multiple regression analyses focused on examining potential impacts of POPs on southern sea otters. Management decisions made with respect to this population should be based on evaluating large sample sizes and broad, non-biased sample distributions that encompass the full diversity of the southern sea otter population within California.

Table 24. Comparisons among studies of POPs in sea otter livers.

				Summarized test results, mean ng/g wet weight									Associations with cause of death		
Study	Where	Year of Otter Death	N	Sum PCBs	Sum PBDEs	Sum DDTs	pp-DDE	Dieldrin	Sum chlordanes	Sum butyltins	PFOS	Various	Examined ?	Data stratified for sex, age and nutritional condition?	Conclusions for cause of death
Shaw, 1971	CA	1969-1970	8	NR	NR	2888	NR	NR	NR	NR	NR	NR	No	N/A	N/A
Nakata et al., 1998	CA	1970	20	1600	NR	1802	NR	NR	93	NR	NR	NR	Yes	No	Otters dying due to infectious disease and miscellaneous causes had higher concentrations of PCBs and DDTs. However, ranges were wide and this finding was not significant for PCBs. DDTs were significantly higher in otters dying due to infectious disease, versus those dying from trauma or unknown cause.
Bacon et al., 1999	Aleutian Islands	1988-1992	7	310	NR	36	36	3	NR	NR	NR	NR	No	N/A	N/A
"	SE AK	1988-1992	9	8	NR	1	1	2	NR	NR	NR	NR	No	N/A	N/A
"	CA	1988-1992	7	190	NR	850	840	1	NR	NR	NR	NR	No	N/A	N/A
Kannan et al., 1998	CA	1992-1996	35	NR	NR	NR	NR	NR	NR	1090	NR	NR	Yes	No	Sea otters with infectious disease had higher liver butyltin concentrations than those dying from trauma & other causes.
Kannan et al., 2006	CA	1992-2002	80	NR	NR	NR	NR	NR	NR	NR	55	60	Yes	No	Significantly increased concentrations of PFOA & PFOS were present in otters dying due to infectious disease
Kannan et al., 2006	CA	1992-2002	80	NR	NR	NR	NR	NR	NR	NR	NR	Various	Yes	No	Concentrations of Mn, Co, Zn and Cd were elevated in the diseased and emaciated sea otters relative to the "other" group.
Kannan et al., 2007	CA	1992-2002	80	624	83	NR	NR	NR	NR	NR	NR	NR	Yes	No	PBDEs did not vary by time, region or disease state and PCBs varied only by region, with higher concentrations south of Cayucos than between Seaside and Cayucos. PBDEs and PCBs were higher in emaciated otters.
Current Study	CA	2000-2005	227	177	48	635	621	7	21	36	NR	NR	Yes	Yes	No associations were detected with summed POPs for any disease group; Increased concentrations of some analytes were associated with a higher risk of death due to trauma, bacterial infection, acanthocephalan peritonitis and protozoal disease; No associations were found with summed or individual POP concentrations and pooled causes of infectious disease

NR = Not reported.
N/A = Not available

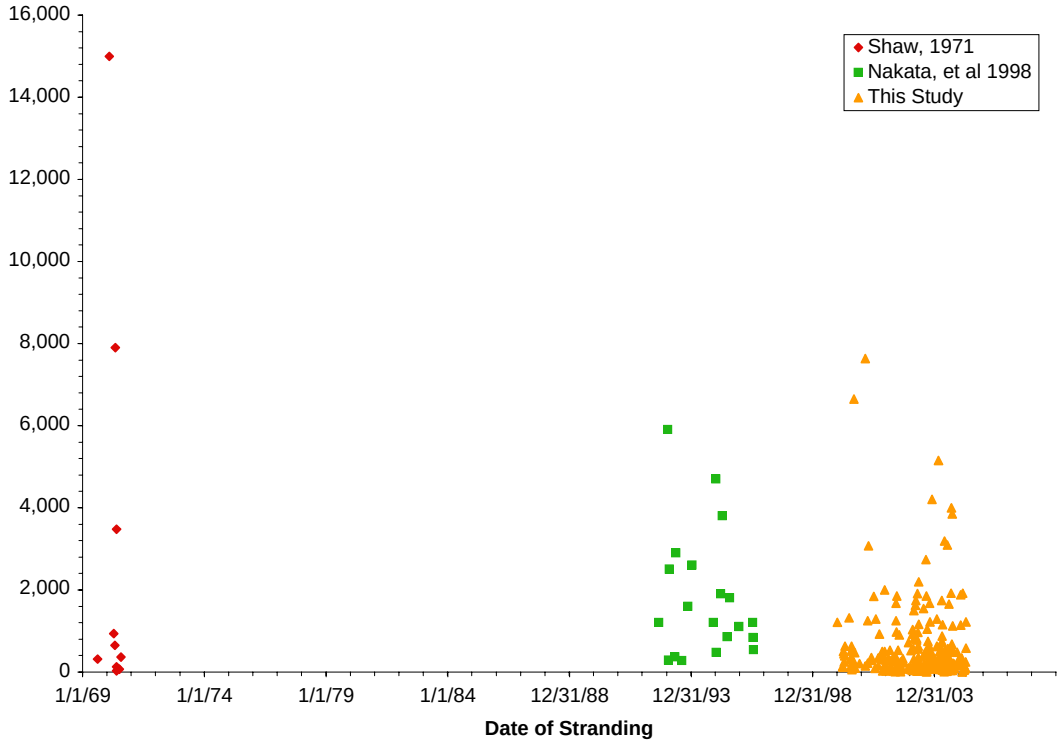

Figure 20. Replicate values for three studies of DDTs in sea otter livers.

Some potential explanations for the absence of a clear pattern between exposure to the majority of POPs and an enhanced risk of sea otter death due to infectious disease in the current study include the following: 1) Because the top 3 causes of sea otter death were included in all analyses, and some sea otters had >1 major category of disease present at necropsy, our ability to detect significant differences in risk of POP exposure by disease group could have been diluted by including data from the same otter in >1 risk group; 2) Because so many different variables were considered, the statistical analyses, the probability of detection of significant effects due to any single variable is low, unless those effects are marked; thus more subtle POP-associated effects could easily be missed; 3) Effects due to one or more of the tested POPs could be synergistic, and thus could not accurately be detected via the current study design, or 4) There is minimal enhanced risk due to increased liver burdens for the majority of POPs in southern sea otters and the observed data are accurate.

The techniques used to complete the current study differed substantially from those of prior studies (Table 24), so any direct comparisons must be made with caution. Some key differences include; 1) The present study employed larger sample sizes (n = 227) than previous studies, thus permitting more rigorous data stratification and statistical analyses; 2) Case selection in the present study was based solely on the date of otter stranding, without regard to each otter's location, age class (excluding pups), sex or cause of death. This means that all qualifying liver samples in each age class were included without pre-test selection bias for potential high and low-risk coastal areas for POP exposure; 3) Enrolled otters were "blind-scored" for a number of

attributes, lesions and potential risk factors by a veterinary pathologist and collaborators prior to completion of liver POP testing, to minimize risk of selection bias; 4) Univariate and multivariate statistical analyses included adjustment of the odds ratios for sea otter death relative to POP exposure by weighing the contributions of other significant risk factors, such as age, sex and nutritional condition 5) Univariate and multivariate statistical analyses were completed for 5 different major causes of sea otter death, as well as for otter death due to all infectious diseases pooled as a single group; 6) Statistical analyses were completed for both the summed pollutants in each major group (e.g., total PBDEs) and for 138 specific POP analytes (e.g., PBDE 28); 7) Because southern sea otters often die with >1 major disease process at necropsy, both the primary and top two contributing causes of death were considered in all analyses, to facilitate broad comparisons between major (and often concurrent) processes present in each animal and liver POP concentrations; 8) Groupings for the major disease or death categories, case selection criteria and extent of data stratification differed among the various studies (Table 24). 9) Sample collection time points and sample distributions across the sea otter range varied between studies 10) In some cases, different tests were used for POP measurement or data analysis and 11) Of necessity, a different laboratory was used for completion of POP testing in the present study.

Summary and Conclusions

Here we present the results of a multi-year epidemiological study focused on examining associations between liver concentrations of persistent organic pollutants and major causes of death in threatened southern sea otters. The overall findings from this study indicate that some POPs contribute to the risk of sea otter death due to specific infectious disease or trauma. However, our data suggest that several demographic, spatial and environmental factors contribute as strongly, or more strongly to the risk of otter death due to trauma or infection than do POP liver burdens. In addition, when all causes of infectious disease were pooled into a single multivariate model and stratified with respect to age, nutritional condition and other factors, risks due to higher liver concentrations of specific POP analytes were no longer statistically significant. Taken together, these preliminary findings suggest that sea otter exposure to anthropogenic pollutants, although important, are not the primary driving force causing threatened southern sea otter declines in central California. In addition, these data suggest that concurrent demographic, spatial and environmental risk factors contribute very significantly to the risk of sea otter mortality and with respect to liver POP concentrations. Therefore, these factors must also be carefully considered in any studies attempting to assess disease risks associated with environmental POP exposure. Some additional steps that should be performed include testing available liver samples from time-matched sea otter pups and testing cryopreserved milk samples so that some inferences can be made regarding the relative importance of transplacental and lactational transfer of liver POP burdens in sea otter pups, when compared with older otters that are actively foraging. Prior studies suggest that a large proportion of POP tissue loading in marine mammals occurs as a result of transplacental and lactational transfer (Wells et al., 2005, Metcalf et al., 2004, Nakata et al., 1998, Tanabe et al., 1997). Our next critical step is to repeat these statistical analyses using data groupings that more closely approximate those of previous studies, including conversion of POP test results to categorical variables (e.g., low, medium and high, and present or absent), further stratifying the causes of death and considering only the major cause of death for each enrolled otter, instead of pooling the top 3 major findings, as was done for the present study. Until these analyses are completed,

comparisons with prior studies examining associations of POP tissue levels with categorical causes of death in sea otters must be made with extreme caution.

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APPENDIX A.
Conventional analytes and POPs analyzed in sea otter tissues.

Conventional
Moisture
Lipid
Persistent Organic Pollutants[a]
PAHs
1-Methylnaphthalene
2,3,5-Trimethylnaphthalene
2,6-Dimethylnaphthalene
2-Methylnaphthalene
1-Methylfluorene
2-Methylfluoranthene
4-Methyldibenzothiphene
Biphenyl
Naphthalene
1-Methylphenanthrene
3,6-Dimethylphenanthrene
Acenaphthene
Acenaphthylene
Anthracene
Fluorene
Phenanthrene
Benz(a)anthracene
Chrysene
Fluoranthene
Pyrene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(e)pyrene
Benzo(k)fluoranthene
Dibenz(a,h)anthracene
Perylene
Benzo(ghi)perylene
C1-Chrysenes

C2-Chrysenes
C3-Chrysenes
Indeno(1,2,3-cd)pyrene
Dibenzothiophene
C1-Dibenzothiophenes
C2-Dibenzothiophenes
C3-Dibenzothiophenes
C1-Fluoranthene/Pyrenes
C1-Fluorenes
C2-Fluorenes
C3-Fluorenes
C1-Naphthalenes
C2-Naphthalenes
C3-Naphthalenes
C4-Naphthalenes
C1 Phenanthrene/Anthracenes
C2-Phenanthrene/Anthracenes
C3-Phenanthrene/Anthracenes
C4-Phenanthrene/Anthracenes
Pesticides
Cyclopentadienes
Aldrin
Dieldrin
Endrin
Chlordanes
cis-Chlordane
trans-Chlordane
cis-Nonachlor
trans-Nonachlor
Heptachlor
Heptachlor Epoxide
Oxychlordane
DDTs
o,p'-DDD
o,p'-DDE
o,p'-DDT

p,p'-DDD
p,p'-DDE
p,p'-DDT
HCH
alpha-HCH
beta-HCH
delta-HCH
gamma-HCH
Other
Chlorpyrifos
Dacthal
Diazinon
Endosulfan I
Endosulfan II
Endosulfan Sulfate
Mirex
Oxadiazon
Hexachlorobenzene
PCB congeners
8, 18, 27, 28, 29, 31, 33, 44, 49, 52, 56, 60, 66, 70, 74, 87, 95, 97, 99, 101, 105, 110, 114, 118, 128, 137, 138, 141, 149, 151, 153, 156, 157, 158, 170, 174, 177, 180, 183, 187, 189, 194, 195, 200, 201, 203, 206, 209
PBDE congeners
17, 28, 47, 66, 85, 99, 100, 138, 153, 154, 183
Organotins
Monobutyltin
Dibutyltin
Tributyltin