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Immunomodulatory effects of organochlorine mixtures upon in vitro exposure of peripheral blood leukocytes differ between free-ranging and captive southern sea otters (*Enhydra lutris*)

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Abstract

Organochlorines (OCs), notably polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), are ubiquitous environmental contaminants. Contaminant-induced immunosuppression by OCs has been implicated as a co-factor in the deaths of thousands of marine mammals in infectious disease epizootics over the last two decades, and limited studies support the hypothesis that PCBs are immunomodulatory. This study represented a unique opportunity to assess the potential differences in susceptibility to OCs between captive and free-ranging sea otters originating from the same genetic population. In vitro immune assays were utilized to evaluate both innate (phagocytosis and respiratory burst) and acquired (mitogen-induced B and T lymphocyte proliferation) immune functions. Individual PCBs (138, 153, 169 and 180) as well as TCDD and all 26 possible combinations were tested. Mixtures were tested as they represent 'real life' exposure. Our results suggest that (1) different immune functions were sensitive to different OC mixtures in both magnitude and direction (enhancement/suppression) and (2) differences in sensitivities upon in vitro exposure to OCs occurred between free-ranging and captive otters. Differences in susceptibility could be explained by the acute stress of capture, the chronic stress of captivity or nutritional differences. Understanding differences in toxicity to different populations of sea otters will have important implications for risk assessment as well as conservation and management strategies.

Keywords: Southern sea otter; Organochlorine; Immunotoxicology

1. Introduction

Southern sea otters (*Enhydra lutris*) inhabit approximately 250 miles of the central California coastline and were listed as threatened by the US Fish and Wildlife Service in 1977. Despite protection and population growth from the 1980s and early 1990s, this population has experienced no significant population growth since 1994 (Jessup et al., 2004). This has been associated with high adult mortality with common causes of death including protozoal encephalitis, acanthocephalan-related disease, shark attack and cardiac disease (Kreuder et al., 2003). Another potential factor that

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may contribute to the decline of sea otters may include contaminant-induced immunotoxicity upon exposure to environmental chemicals such as organochlorines (OCs), a likely contributing factor in the morbillivirus-attributable deaths of tens of thousands cetaceans and pinnipeds (Kannan et al., 1993; Ross et al., 1996; Troisi et al., 2001; Van Loveren et al., 2000).

Environmental OCs, including polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), are ubiquitous contaminants and have been measured in the tissues of marine mammals throughout the world (Andersen et al., 2001; Hutchinson and Simmonds, 1994; Krahn et al., 1997; Lake et al., 1995; Norstrom et al., 1998; Severinsen et al., 2000; Troisi et al., 2001). In southern sea otters, OCs have been detected in liver, kidney and brain tissues and at levels significantly higher than sea otters from Southeast Alaska (Bacon et al., 1999; Kannan et al., 2004). OCs have also been detected in serum of live southern sea otters at levels 50–100 times higher than sea otters in more pristine areas of Alaska (Jessup et al., unpublished).

The immune system represents the interface between an individual's health and the pathogens present in its environment. Proper functioning of the innate and adaptive arms of the immune system is essential in preventing or eliminating disease. Multiple studies have demonstrated that OCs are associated with changes in both innate and adaptive immune functions in several marine mammal species, including sea otters (De Guise et al., 1998; de Swart et al., 1996; Lahvis et al., 1995; Levin et al., 2005a, 2004, 2005b; Mori et al., 2006). Causal relationships between contaminant exposure and immunotoxicity have been demonstrated in American mink (*Mustela vison*), a surrogate species for sea otters (Schwartz et al., 2004a,b).

The present study provided a unique opportunity to compare the immunomodulatory effects of OCs upon in vitro exposure between two populations of southern sea otters from the same genetic stock. The in vitro effects of OCs on phagocytosis, an innate immune functions necessary to engulf extracellular pathogens, and T cell-induced lymphocyte proliferation, an adaptive immune function leading to the production of effector and memory T cells, have previously been documented in free-ranging southern sea otters (Levin et al., 2005b; Mori et al., 2006). The current study measured the immunomodulatory effects of OCs on phagocytosis and T cell-induced proliferation in captive sea otters as well as two additional immune functions, the respiratory burst, an innate immune function necessary to destroy engulfed pathogens, and B cell-induced lymphocyte proliferation, an adaptive immune function leading to the production of effector and memory B cells, in both captive and free-ranging southern sea otters. The effects of OCs on all immune functions and the potential differences between captive and free-ranging sea otters in their susceptibility to the effects of OCs are discussed. Understanding the risk for immunotoxicity upon exposure to OCs in different populations of southern sea otters will have important implications for risk assessment as well as conservation and management strategies.

2. Materials and methods

2.1. Animals and blood sampling

Blood samples from captive and free-ranging southern sea otters (*E. lutris*) were collected from the California Department of Fish and Game, Marine Wildlife Veterinary Care and Research Center, Santa Cruz, CA and the Monterey Bay Aquarium, Monterey, CA. All animals were either sub-adult or adult, of both sexes, and clinically healthy based on a veterinary examination and complete blood count and serum chemistry panel values within normal ranges. Free-ranging sea otters were captured as part of ongoing biological telemetry and tagging studies of sea otter ecology and health. Captive sea otters were all animals that had been declared ''non-releasable'' by USFWS and held under appropriate permits. All blood samples for our studies taken sterilely from the jugular vein were collected into sodium heparin tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), kept cool on ice packs in the dark, shipped overnight and processed within 24 h.

2.2. Organochlorines

PCBs 138, 153, 169, 180 (purity >98.4%) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, purity >98%) were purchased from Ultra Scientific (North Kingston, RI). PCBs were re-suspended in endotoxin-free dimethyl sulfoxide (DMSO, Sigma, St Louis, MO) to prepare stock solutions. OCs were then added to Dulbecco's modified eagle medium (DMEM, Gibco BRL, Grand Island, NY) supplemented with (all from Gibco BRL) 1 mM sodium pyruvate, 100 μM non-essential amino acids, 25 mM HEPES, 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml) and 10% fetal bovine serum (Hyclone, Logan, UT) to prepare working solutions. Non-coplanar PCBs 138, 153 and 180 were tested as they are among

the most abundant in southern sea otters and other species of marine mammals (Kannan et al., 2004; Martineau et al., 1987). The coplanar PCB 169 and TCDD were chosen for their known toxicity. Single compounds as well as all possible mixtures of 2, 3, 4 and all five OCs were tested. The final concentration of each PCB (5 ppm) and TCDD (0.05 ppb) tested, alone or in a mixture, represented concentrations previously used to investigate and compare the immunotoxic effects of OCs upon in vitro exposures in several marine mammal species (Levin et al., 2007, 2004, 2005b; Mori et al., 2006). The final DMSO concentration did not exceed 0.4%.

2.3. Isolation of leukocytes

Isolation of leukocytes was performed prior to the evaluation of all immune functions. For phagocytosis and the respiratory burst, erythrocytes were lysed using NH_4Cl and the leukocytes were re-suspended in Hanks Balanced Salt Solution (HBSS, Gibco BRL). Cells were washed twice with HBSS, and their viability was assessed using the exclusion dye trypan blue. Viability was typically greater than 90%. For lymphocyte proliferation, peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation on Ficoll-Paque plus (Amersham Biosciences, Uppsala, Sweden) for 35 min at 990 × *g*. The PBMC were re-suspended in complete DMEM, washed twice and enumerated with their viability assessed using the exclusion dye trypan blue. Viability was typically greater than 90% and never fell below 85% in leukocyte samples used for experiments.

2.4. Phagocytosis

Phagocytosis was evaluated in vitro as previously described (Levin et al., 2005b). Briefly, the leukocyte concentration was adjusted to 2×10^6/ml in HBSS, followed by incubation at 37 °C with 5% CO_2 with the toxicant mixtures for 3 h in round bottom 96-well plates (32 treatments per individual animal, each in a single well, up to three individual animals per plate) (Falcon, Becton Dickinson, Lincoln Park, NJ). One micrometer-diameter fluorescent latex beads (Molecular Probes, Eugene, OR) were added to the cell suspension to obtain a ratio of approximately 100 beads/cell, and cells were incubated for 1 additional hour at 37 °C, under agitation at 300 rpm using a Thermomixer R (Eppendorf, Hamburg, Germany). The cell suspension from each well was then layered on a cushion of ice cold 3% bovine serum albumin (Sigma) and centrifuged at 150 × *g* for 8 min at 4 °C. The supernatant containing the free beads was discarded and the cells were re-suspended in 200 μl of phosphate buffered saline (PBS, Gibco) containing 1% neutral buffered formalin (Decal Corp., Tallman, NY). Cells were stored at 4 °C until analysis (within 24 h).

2.5. Respiratory burst

The respiratory burst was evaluated in vitro as previously described (Levin et al., 2007). Briefly, the leukocyte concentration was adjusted to 2×10^6/ml in HBSS. Cell suspensions were incubated for 30 min at 37 °C, in the dark, with 5 μM of 2,7-dichlorofluorescin diacetate (DCFDA, Molecular Probes), a probe used to quantify the production of H_2O_2. The cell suspension was centrifuged for 10 min at 220 × *g* and re-suspended in PBS-G. For each individual animal, leukocytes were plated with the different mixtures of toxicant in each of two round bottom 96-well plates (Falcon, Becton Dickinson). 10^{-9} M phorbol myristate acetate (PMA, Molecular Probes), a cell activator, was added to the first plate and PBS-G (control) was added to the second plate. Both plates were incubated for 1 h at 37 °C. After incubation, cells were fixed with 1% neutral buffered formalin and stored at 4 °C until analysis (within 24 h).

2.6. Flow cytometry

The fluorescence of approximately 10,000 cells was read with a FACScan (Becton Dickinson, Rutherford, NJ) flow cytometer using the CellQuest software (Becton Dickinson Immunocytometry System, San Jose, CA). Neutrophils and monocytes were gated electronically according to their relative size (FSC) and complexity (SCC). For phagocytosis, the fluorescence of the cells was read at 530 nm (FL-1) on a logarithmic scale using the fluorescence of free beads as reference. Cells acquired a fluorescence equal to that of the number of beads they ingested. Phagocytosis was reported as the percentage of cells that had phagocytized one or more beads. The respiratory burst was evaluated as the mean fluorescence at 530 nm (FL-1) of the PMA stimulated cells and reported as the ratio (stimulation index) of the mean fluorescence of PMA stimulated cells divided by the mean fluorescence of the unstimulated cells.

2.7. Lymphocyte proliferation

Mitogen-induced lymphocyte proliferation was evaluated in vitro as previously described (Mori

et al., 2006). Lymphocytes in complete DMEM were plated (2×10^5 cells/well) in 96-well flat bottom tissue culture plates (Falcon, Becton Dickinson, Franklin Lakes, NJ) in triplicate for each chemical mixture. Cells were incubated at 37 °C with 5% CO_2 for a total of 66 h with either the T cell mitogen, concanavalin A (ConA, Sigma) or the B cell mitogen, lipopolysaccharide (LPS, Sigma). ConA and LPS were used at the suboptimal concentration of 0.1 and 0.05 μg/ml, respectively. Suboptimal concentrations were used as they proved more sensitive in detecting immunotoxicity (Mori et al., 2006). Lymphocyte proliferation was evaluated as the incorporation of 5-bromo-2′-deoxyuridine (BrdU), a thymidine analogue, added for the last 18 h of incubation, and further detected with a monoclonal antibody and a colorimetric enzymatic reaction (Cell Proliferation ELISA BrdU (colorimetric), Roche Diagnostics GmbH, Mannheim Germany) as per manufacturer's instructions using an ELISA plate reader (Multiskan EX v.1.0) at 690 nm with a reference wavelength of 450 nm.

2.8. Statistics

Data from seven and five independent phagocytosis experiments from captive and free-ranging sea otters, respectively, were pooled. Data from eight and seven independent respiratory burst experiments from captive and free-ranging sea otters, respectively, were pooled. Data from nine and five independent lymphocyte proliferation experiments from captive and free-ranging sea otters, respectively, were pooled. All three functional immune assays were not always performed from one individual sea otter due to the limited blood sample volume. Repeat blood samples were obtained from some otters, but were used for different immune assays. A repeated measures one-way analysis of variance (RM ANOVA) with Dunnett's test was used to compare the different experimental groups to the unexposed control group. RM ANOVA was evaluated using the SigmaStat Windows 1.0 (Jandel Scientific, San Rafael, CA) software, using $p < 0.05$ for statistical significance. Statistical power for each experiment was greater than 0.8, as required by the statistical software to assure confidence in the ability to detect changes when they truly existed.

3. Results

3.1. Effects of OCs on phagocytosis

None of the exposures significantly modulated neutrophil phagocytosis from either captive (modified from Levin et al. (2005b) as sample size was increased) or free-ranging sea otters (data not shown). Eight of the 31 exposures significantly reduced monocyte phagocytosis from free-ranging sea otters (modified from Levin et al. (2005b) as sample size was increased), with no effects from captive sea otters (Table 1).

3.2. Effects of OCs on the respiratory burst

Five mixtures significantly reduced neutrophil respiratory burst from captive sea otters, with no effects from free-ranging sea otters (Table 2). Four and two mixtures significantly reduced monocyte respiratory burst from captive and free-ranging sea otters, respectively (Table 2).

Table 1
Effects of OCs on monocyte phagocytosis

	Monocytes	
	Captive $N = 7$	Free-ranging $N = 5$
138	ns	ns
153	ns	ns
169	ns	ns
180	ns	ns
TCDD	ns	ns
138 + 153	ns	↓ 50%
138 + 169	ns	ns
138 + 180	ns	ns
138 + TCDD	ns	ns
153 + 169	ns	ns
153 + 180	ns	↓ 42%
153 + TCDD	ns	ns
169 + 180	ns	ns
169 + TCDD	ns	ns
180 + TCDD	ns	ns
138 + 153 + 169	ns	ns
138 + 153 + 180	ns	ns
138 + 153 + TCDD	ns	ns
138 + 169 + 180	ns	ns
138 + 169 + TCDD	ns	ns
138 + 180 + TCDD	ns	ns
153 + 169 + 180	ns	ns
153 + 169 + TCDD	ns	ns
153 + 180 + TCDD	ns	ns
169 + 180 + TCDD	ns	ns
138 + 153 + 169 + 180	ns	↓ 77%
138 + 153 + 169 + TCDD	ns	↓ 57%
138 + 153 + 180 + TCDD	ns	↓ 43%
138 + 169 + 180 + TCDD	ns	↓ 53%
153 + 169 + 180 + TCDD	ns	↓ 53%
138 + 153 + 169 + 180 + TCDD	ns	↓ 62%

↑, Significant increase ($p < 0.05$) and % change from unexposed control; ↓, significant increase ($p < 0.05$) and % change from unexposed control; ns, not significant ($p > 0.05$); N = number of independent experiements pooled (see Section 2).

Table 2
Effects of OCs on the neutrophil and monocyte respiratory burst

	Neutrophils		Monocytes	
	Captive $N = 8$	Free-ranging $N = 7$	Captive $N = 8$	Free-ranging $N = 7$
138	ns	ns	ns	ns
153	ns	ns	ns	ns
169	ns	ns	ns	ns
180	ns	ns	ns	ns
TCDD	ns	ns	ns	ns
138 + 153	ns	ns	ns	ns
138 + 169	ns	ns	ns	ns
138 + 180	ns	ns	ns	ns
138 + TCDD	ns	ns	ns	ns
153 + 169	ns	ns	ns	ns
153 + 180	ns	ns	ns	ns
153 + TCDD	ns	ns	ns	ns
169 + 180	ns	ns	ns	ns
169 + TCDD	ns	ns	ns	ns
180 + TCDD	ns	ns	ns	ns
138 + 153 + 169	ns	ns	ns	ns
138 + 153 + 180	ns	ns	ns	ns
138 + 153 + TCDD	ns	ns	ns	ns
138 + 169 + 180	ns	ns	ns	ns
138 + 169 + TCDD	ns	ns	ns	ns
138 + 180 + TCDD	ns	ns	ns	ns
153 + 169 + 180	ns	ns	ns	ns
153 + 169 + TCDD	ns	ns	ns	ns
153 + 180 + TCDD	ns	ns	↓ 34%	ns
169 + 180 + TCDD	ns	ns	ns	ns
138 + 153 + 169 + 180	ns	ns	ns	ns
138 + 153 + 169 + TCDD	↓ 26%	ns	ns	ns
138 + 153 + 180 + TCDD	↓ 30%	ns	↓ 32%	ns
138 + 169 + 180 + TCDD	↓ 38%	ns	ns	↓ 29%
153 + 169 + 180 + TCDD	↓ 30%	ns	↓ 39%	ns
138 + 153 + 169 + 180 + TCDD	↓ 23%	ns	↓ 32%	↓ 26%

↑, Significant increase ($p < 0.05$) and % change from unexposed control; ↓, significant increase ($p < 0.05$) and % change from unexposed control; ns, not significant ($p > 0.05$); N = number of independent experiements pooled (see Section 2).

3.3. Effects of OCs on lymphocyte proliferation

Six mixtures significantly increased ConA-induced lymphocyte proliferations from free-ranging sea otters (Mori et al., 2006), with no effects from captive otters (Table 3). LPS-induced lymphocyte proliferation was not affected by exposure to OC mixtures in either captive or free-ranging sea otters (data not shown).

4. Discussion

Sea otter innate and adaptive immune functions were not equally modulated upon exposure to OCs. In all experiments, for each individual, the functions of the cells that were exposed in vitro were measured and compared to those of unexposed cells from the same individual. In other words, cells that were unexposed in vitro represented the baseline for that individual and served as an intrinsic control. Therefore, our assays measured changes in immune functions resulting from in vitro exposure irrespective of previous natural exposure to OC.

The mixtures that modulated sea otter monocyte phagocytosis resulted in significant reductions. In previous studies, in vitro exposure to OCs also significantly reduced phagocytosis across a variety of cetacean and pinniped species (Levin et al., 2004, 2005b). The magnitude of the reduction in monocyte phagocytosis in sea otters (between 42 and 77%) was within the same range as in other marine mammals (46–86%) (Levin et al., 2005b). Taken together, these data suggests that sea otters are similar to other marine mammals in their sensitivity to the effects of OCs on

Table 3
Effects of OCs on mitogen-induced (ConA) lymphocyte proliferation

	ConA	
	Captive $N = 9$	Free-ranging $N = 5$
138	ns	ns
153	ns	ns
169	ns	ns
180	ns	ns
TCDD	ns	ns
138 + 153	ns	↑ 48%
138 + 169	ns	ns
138 + 180	ns	ns
138 + TCDD	ns	ns
153 + 169	ns	↑ 63%
153 + 180	ns	↑ 72%
153 + TCDD	ns	ns
169 + 180	ns	ns
169 + TCDD	ns	↑ 60%
180 + TCDD	ns	↑ 67%
138 + 153 + 169	ns	ns
138 + 153 + 180	ns	ns
138 + 153 + TCDD	ns	↑ 89%
138 + 169 + 180	ns	ns
138 + 169 + TCDD	ns	ns
138 + 180 + TCDD	ns	ns
153 + 169 + 180	ns	ns
153 + 169 + TCDD	ns	ns
153 + 180 + TCDD	ns	ns
169 + 180 + TCDD	ns	ns
138 + 153 + 169 + 180	ns	ns
138 + 153 + 169 + TCDD	ns	ns
138 + 153 + 180 + TCDD	ns	ns
138 + 169 + 180 + TCDD	ns	ns
153 + 169 + 180 + TCDD	ns	ns
138 + 153 + 169 + 180 + TCDD	ns	ns

↑, Significant increase ($p < 0.05$) and % change from unexposed control; ↓, significant increase ($p < 0.05$) and % change from unexposed control; ns, not significant ($p > 0.05$); N = number of independent experiements pooled (see Section 2).

phagocytosis, particularly monocyte phagocytosis, and to similar magnitudes.

The mixtures that modulated the sea otter neutrophil and monocyte respiratory burst resulted in significant reductions. In a previous study, the neutrophil and monocyte respiratory burst was significantly enhanced in several cetaceans species, while the monocyte respiratory burst was significantly reduced in harbor seals (Levin et al., 2007). The magnitude of the reduction in the harbor seal monocyte respiratory burst (33–67%) was higher than in sea otters (23–39%). Taken together, these data suggest that sea otters are similar to other marine mammals in their sensitivity to the effects of OCs on the respiratory burst, but not always in the same direction or to the same magnitude.

The OC mixtures that modulated sea otter T cell proliferation resulted in significant increases. In a previous study, T cell proliferation was also significantly increased in several marine mammals (Mori et al., 2006), with the magnitude of the change approximately within the median range. B cell proliferation was not modulated in the current study. In previous experiments, B cell proliferation was also not modulated in beluga whales, whereas several other marine mammals were sensitive to several mixtures, resulting in significant increases in proliferation (C. Mori, unpublished data). Taken together, these data suggest that not all marine mammals are sensitive to the effects of OCs on lymphocyte proliferation. In those species that were sensitive, the lymphocyte proliferation was increased, but not always to the same magnitude.

In general, innate immune functions appeared to be reduced whereas adaptive immune functions appeared to be increased. However, any contaminant-related modulation of these immune functions is of concern. Acanthocephalan infestation and *Toxoplasma gondii* and *Sarcocystic neurona* encephalitis are three of the leading causes of death in southern sea otters (Kreuder et al., 2003). OC-induced immumomodulation may render sea otters more susceptibility to these infections. A reduction in phagocytosis could result in decreased clearance of unwanted cells or extracellular pathogens (Kuby, 1997). In addition, if monocyte functions are impaired after cells mature into tissue macrophages, the adverse effects may extend to the adaptive immune system through defects in antigen presentation. A reduction in the respiratory burst could result in the reduction in the killing of pathogens engulfed through phagocytosis (Kuby, 1997). In humans, the most notable defect associated with the inability of neutrophils to produce hydrogen peroxide during the respiratory burst is chronic granulamatous disease (CGD) (Roos et al., 2003).

The increase in lymphocyte proliferation with cells exposed to OC represents a change from cells undergoing baseline lymphocyte proliferation (cells exposed to mitogens, but not OCs). A ‘positive’ stimulation of the immune system should result in the recognition of a pathogen and the mounting of an effective immune response (i.e., generation of effector and memory B and T cells) to eliminate the pathogen. Non-specific and uncontrolled stimulation of lymphocyte proliferation, induced by OCs, may not lead to the elimination of the pathogen. If fact, it could result in serious consequences. Naive T cells require two distinct signals for activation, proliferation and subsequent differentiation

into effector and memory cells. A continuous stimulation by OCs could mimic signal one, and without the proper second signal (either T cell receptor-CD3 complex or CD28-B7 interactions), may lead to a state of anergy, an active state of unresponsiveness in which cells remain alive but unable to mount a functional immune response (Schwartz, 2003). Chronic, low level OC stimulation could result in a reduced threshold for survival/proliferation, which could in turn lead to the development autoimmunity or cancer.

Captive and free-ranging sea otters differed in their susceptibility to the immunomodulatory effects of OCs. Free-ranging sea otters were more susceptible to changes in phagocytosis and lymphocyte proliferation while captive sea otters were more susceptible to changes in the respiratory burst. There are several potential explanations for the differences in susceptibility between the two populations. First, each population is exposed to different types of stress. For free-ranging otters, the physical stress due to capture (tagging and implantation studies) could stimulate the hypothalamic-pituitary-adrenal (HPA) axis resulting in elevated adrenal hormones (i.e., cortisol, aldosterone), which may directly and/or indirectly modulate the immune system (Doenhoff and Leuchars, 1977; Ishizaka et al., 1980). Serum cortisol levels were significantly increased in southern elephant seal pups 5–35 min post-capture (Engelhard et al., 2002) however, the relatively short capture and restraint times did not induce a significant neuroendocrine stress response in wild caught bottlenose dolphins (Ortiz and Worthy, 2000). The effects of capture stress and hormone levels in free-ranging sea otters are not completely understood. Other potential stressors include predator stress (exertion to out run potential shark attacks), reproductive stress (females either caring for newborns or gestating) and nutritional stress or prey specialization (lack of adequate amounts of or nutrient rich prey species). In addition, free-ranging sea otters are potentially exposed to different environmental contaminants (via diet or oil spills) (Bacon et al., 1999; Nakata et al., 1998) and pathogens (Kreuder et al., 2003). Free-ranging sea otter are much more likely to live in environments polluted with man-made chemicals such as polychlorinated biphenyls, legacy pesticides such as DDT and its breakdown products, petroleum products, biotoxins such as domoic acid and/or pathogens such as *T. gondii*.

Sea otters in captivity would presumably experience different stressors (i.e., no predation or nutritional stress) but may experience stress associated with the chronic state of captivity. In cheetahs, for example, the stress hormone cortisol was significantly higher in captive cheetahs than in free-ranging cheetahs (Terio et al., 2004). The effects on long-term captivity on sea otter immune functions and their sensitivity to immunotoxicants have not been established.

Although differences in stress may account for the differences in the susceptibility to OCs between the two populations of sea otters, it is important to note that in all experiments, cells were isolated from whole blood prior to their exposure to OCs. Therefore, the direct effects of hormones on immune functions should have been eliminated. Differences at the cell and molecular level would then explain the differences between the two populations. It is possible that pre-exposure of immune cells to hormones (such as cortisol) would have modulated cell signaling pathways and/or gene expression which in turn altered the cells responses to subsequent OC exposure in vitro. This may explain the different responses between captive and free-ranging sea otters. The effects PCB 126 on IL-1β gene expression were affected by pre-incubation of primary cultures of rainbow trout anterior kidney cells with cortisol (Quabius et al., 2005).

There are many factors that may influence the susceptibility of sea otters to the immunotoxicity of OCs. Future studies could assess the influence of the acute stress by assessing the influence of short-term (in vitro) exposure to stress hormones (adrenalin, corticosteroids) on the sensitivity to the immunotoxicity of OCs upon in vitro exposure. The influence of predator and nutritional stress may be more difficult as live captive studies would need to be performed. To the authors' knowledge, this is the first report to demonstrate differences in susceptibility to toxicants between captive and free-ranging animals. Importantly, elucidating the effects of OCs in one population does not necessarily predict the effects in other populations. In terms of risk assessment and management, it will be necessary to understand the effects of OCs, as well as other environmental contaminants, in various populations of sea otters to better direct local conservation and management efforts.

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