# Organochlorine Contaminants in Blubber from Stranded Marine Mammals Collected from the Northern Oregon and Southern Washington Coasts; Implications for Re-introducing California Condors, *Gymnogyps californianus*, in Oregon

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**ABSTRACT**

Re-introduction of California condors into Oregon is currently being considered, but there are concerns about the safety of potential food sources of this species. Condors are opportunistic feeders and a largely available food source for this species will be stranded marine mammal carcasses. We analyzed 37 blubber samples from 7 different marine mammal species collected from the Oregon and Southern Washington coasts for 18 OC pesticides and 16 PCBs. DDE was the most prevalent OC contaminant, making up more than 58% of the total OC concentration measured. There were no significant differences in OC content between species or sexes.

**KEY WORDS**

Marine mammals • DDE • PCBs

Oregon Zoo, Portland, OR, in partnership with the U.S. Fish and Wildlife Service Condor Recovery Team, has developed a successful captive condor breeding facility in Oregon that has become the second largest of the four condor breeding facilities in the U.S. There are concerns in all the release areas about the safety of the potential food sources of this species. For example, establishment of viable populations in the wild is currently being hampered by lead contamination when condors fed on carcasses of animals that were shot with lead bullets or lead shot (Finkelstein et al. 2012). As condors are opportunistic feeders, a principle food source for reintroduced condor populations in the Pacific Northwest will likely be the carcasses of stranded marine mammals (Walters et al. 2008). In Northern Oregon and Southern Washington we have observed that large avian scavengers (e.g., American bald eagles) consume all flesh on the carcasses, often including substantial blubber layers. Due to the life history and trophic status of the marine mammal species found stranded on local beaches in Oregon and Washington and in the Columbia River, it is possible that condors feeding on them could accumulate persistent organochlorine (OC) contaminants. Numerous studies have shown that marine mammals from the Pacific coast of North America have levels of Dichlorodiphenyldichloroethylene (DDE) and polychlorinated biphenyls (PCBs) that may affect the health of the marine mammals and/or the health of scavengers that feed on the carcasses (Subramanian et al., 1987, Blasius and Goodmanlowe 2008). Recent assessment of contaminants in pinnipeds commonly found dead on the beaches of Southern California demonstrated the presence of extremely high levels of DDE and PCBs in California sea lions and harbor seals (Blasius and Goodmanlowe 2008). These values significantly exceed those known to impair immune, reproductive, developmental and endocrine systems in harbor seals (de Swart et al. 1996).

In the past, there were concerns over the decline in condor populations due to eggshell thinning in DDE exposed birds (Snyder and Meretsky 2003) and currently, the relationship between DDE exposure and reproductive failure of wild condors which are feeding on beached marine mammals in Central California is being investigated (Burnett et al. *in press*). However, an earlier study of stranded marine mammals in Oregon reported much lower concentrations of these contaminants in most of the commonly stranded species (Hayteas and Duffield 1997).

The aim of this study was to screen blubber samples taken from species of marine mammals commonly stranded in Oregon for OC pesticides and PCBs. in order to determine if consumption of these carcasses poses a potential risk for re-introduced condors or other avian scavengers. In addition, we were also interested in whether any relationship existed between pesticide levels in marine mammal blubber samples, sex and disease status.

**Materials and Methods**

Blubber samples from 37 marine mammals collected by the Northern Oregon Southern Washington Marine Mammal Stranding Network from 2007 through 2010 were examined: 8 California sea lions (*Zalophus californianus*), 8 Steller sea lions (*Eumetopias jubatus*), 8 harbor seals (*Phoca vitulina*), 10 harbor porpoises (*Phocoena phocoena*) and one sample each from a sperm whale (*Physeter macrocephalus*), a Dall's porpoise (*Phocoenoides dalli*) and an elephant seal (*Mirounga angustirostris*). The strandings were concentrated near the north and south of the mouth of the Columbia River, Oregon. Blubber was removed from the dorso-lateral or ventral subscapular region, wrapped in foil and placed on ice. Samples were stored at $-20^{\circ}$C until analysis. Morphological data taken during evaluation of the stranding included sex, length, age, decomposition code, gross necropsy findings and histopathology, if the carcass was fresh.

Blubber samples were analyzed for 18 OC pesticides and 16 PCB congeners (Table 1). Analysis of PCB congeners in blubber samples were selected based on their toxicological significance and their prevalence in environmental samples (McFarlane and Clarke 1989). Lipid extraction and cleanup procedures for blubber samples were done based on procedures described by Feist et al. (2005). Skin was removed from blubber samples and homogenized using a Brinkman Polytron tissue homogenizer. Subsamples of blubber homogenates (approximately 5 g) were combined with anhydrous sodium sulfate and ground into a fine powder using a mortar and pestle. Dried blubber homogenates were Soxhlet extracted for 10 hours using spectral grade petroleum ether and hexane (1:1 vol/vol). Lipid extracts were concentrated using a rotary evaporator, followed by evaporation using a stream of pure nitrogen. Lipid content was determined gravimetrically. Lipid extracts were cleaned up using columns packed with florisil (20 g), and PCBs and chlorinated pesticides were eluted using 6% ethyl ether/petroleum ether (vol/vol). PCBs were separated from OC pesticides using columns packed with silica (5 g), with the PCBs and DDE eluted with hexane (first fraction) and the remaining chlorinated pesticides (second fraction) eluted with hexane/diethyl ether (3:1 vol/vol). Elution volumes were determined by running PCB and pesticides standards through columns and collecting fractions to determine elution patterns.

**Table 1.** Chlorinated pesticides and PCBs measured in marine mammal blubber samples collected from the Oregon and Washington coasts.

| Chlorinated Pesticide | PCB (IUPAC no.) |
|---|---|
| Aldrin | 3,3',4,4'-Tetrachlorobiphenyl (77) |
| α-BHC | 3,3',4,4',5-Pentachlorobiphenyl (126) |
| β-BHC | 3,3',4,4',5,5'-Hexachlorobiphenyl (169) |
| γ-BHC | 2,3,3',4,4'-Pentachlorobiphenyl (105) |
| δ-BHC | 2,2',4,4',5-Pentachlorobiphenyl (118) |
| p,p'DDD | 2,2',3,3',4,4'-Hexachlorobiphenyl (128) |
| p,p'DDE | 2,2',3,4,4',5,5'-Hexachlorobiphenyl (138) |
| p,p'DDT | 2,3,3',4,4',5-Hexachlorobiphenyl (156) |
| Dieldrin | 2,2',3,3',4,4',5-Heptachlorobiphenyl (170) |
| Endrin | 2,2'3,4,5-Pentachlorobiphenyl (87) |
| Endrin aldehyde | 2,2',4,4',5-Pentachlorobiphenyl (99) |
| Endrin ketone | 2,2'4,5,5'-Pentachlorobiphenyl (101) |
| Endosulfan I | 2,2',4,4',5,5'-Hexachlorobiphenyl (153) |
| Endosulfan II | 2,2',3,4,4',5,5'-Heptachlorobiphenyl (180) |
| Endosulfan sulfate | 2,2',3,4,4',5,6-Heptachlorobiphenyl (183) |
| Heptachlor | 2,2',3,3',4,4',5,5'-Octachlorobiphenyl (194) |
| Heptachlor epoxide | |
| p,p'-Methoxychlor | |

PCB and pesticide extracts were analyzed on a Varian CP-3800 gas chromatograph equipped with an electron capture detector, CP-8200 Autosampler, a Star Chromatography Workstation (version 5), and a SPB-608 fused silica capillary column (30 mm x 0.25 mm x 0.25 µm film thickness). The carrier gas was helium (1.5 ml/min), and the makeup gas was nitrogen, with a detector temperature of 300ºC, and an injector temperature of 290 ºC. For each run the oven temperature was set at 150 ºC (4 min) and ramped to 290 ºC (8ºC/min). Quality assurance measures included the analysis of reagent blanks, duplicates, matrix spikes, and surrogate spike samples. Percent recoveries of surrogate spike samples were between 70 and 107%; therefore, samples extracts were not corrected for percent recovery. Approximately 10% of the samples were analyzed as duplicates. Method blanks were analyzed with every 10 samples. The method detection limit for individual PCB congeners and chlorinated pesticides was10 ng/g wet weight and this value was used for the reporting limit.

Interspecies differences in mean contaminant levels were tested by analysis of variance (ANOVA), and a two-tailed, unpaired Student's t test was used to look for differences in contaminant levels between sexes. Significance level was $p \leq 0.05$ for all analyses. Mean values were reported ± SD (standard deviation). All statistics were done using the Statgraphics® (Statistical Graphics, Rockville, MD, USA) statistical software package.

## Results and Discussion

The predominant species of pinnipeds and cetaceans found dead on the beaches in northern Oregon and southern Washington are California sea lions (*Zalophus californianus*), Steller sea lions (*Eumetopias jubatus*), harbor seals (*Phoca vitulina*) and harbor porpoises (*Phocoena phocoena*). These four species alone accounted for approximately 95% of the marine mammal carcasses found in 2007 through 2010 by the Northern Oregon/Southern Washington Marine Mammal Stranding Network, the time period covered by this study. Twenty-six of the stranded mammals had obvious signs of trauma due to human interactions, with seven of these animals confirmed gunshot. Nine of the stranded mammals were noticeably ill (parasitic and bacterial infections or tumors observed on internal organs), with five of these animals being Steller sea lions (Table 2).

**Table 2.** Species (Sp), sex, estimated age, and pesticide levels (µg/g lipid weight) in blubber samples from Steller sea lions (Ej), California sea lions (Zc), harbor seals (Pv), and harbor porpoises (Pp).

| Sp | Sex | Age | DDE | $DDT_{tot}$ | $Pest_{tot}$ | PCB 1a | PCB 1b | PCB 2 | $PCB_{tot}$ |
|---|---|---|---|---|---|---|---|---|---|
| Ej* | F | A | 0.32 | 0.32 | 0.48 | <RL | <RL | <RL | <RL |
| Ej* | F | A | 6.73 | 6.98 | 7.71 | 0.82 | 0.94 | 1.12 | 2.88 |
| Ej* | F | A | 0.20 | 0.20 | 0.55 | <RL | 0.09 | <RL | 0.09 |
| Ej* | F | A | 1.46 | 1.46 | 1.46 | <RL | 0.09 | <RL | 0.09 |
| Ej | F | A | 1.14 | 1.14 | 1.14 | <RL | <RL | <RL | <RL |
| Ej | F | A | 0.56 | 0.56 | 0.56 | <RL | <RL | <RL | <RL |
| Ej* | M | A | 10.03 | 14.72 | 19.11 | 6.29 | 6.90 | 9.01 | 22.20 |
| Ej | M | A | 14.69 | 16.83 | 19.28 | 3.46 | 6.91 | 4.26 | 14.63 |
| Zc | M | A | 16.26 | 19.37 | 20.95 | 6.60 | 1.17 | 1.53 | 9.30 |
| Zc | M | A | 8.62 | 9.36 | 10.36 | 0.93 | 2.07 | 1.24 | 4.25 |
| Zc* | M | A | 34.37 | 40.08 | 45.11 | 11.23 | 7.68 | 5.95 | 24.86 |
| Zc | M | A | 6.53 | 7.02 | 8.65 | 0.24 | 0.51 | 4.25 | 5.00 |
| Zc | M | A | 2.89 | 3.45 | 4.06 | 0.65 | 0.53 | 1.69 | 2.87 |
| Zc | M | A | 1.70 | 1.72 | 1.83 | <RL | 0.24 | 2.10 | 2.34 |
| Zc | M | A | 3.43 | 3.79 | 4.86 | <RL | 0.51 | 1.27 | 1.78 |
| Zc | M | Y | 4.55 | 4.75 | 4.97 | 0.16 | 0.76 | 0.74 | 1.66 |
| Pv | F | SA | 2.23 | 2.41 | 2.51 | <RL | 1.23 | 0.09 | 1.32 |
| Pv | F | A | 12.00 | 15.89 | 23.48 | 4.85 | 4.17 | 6.66 | 15.68 |
| Pv | F | A | 2.33 | 3.00 | 4.31 | 0.76 | 0.16 | 0.99 | 1.91 |
| Pv | M | Y | 3.44 | 3.89 | 4.41 | 1.29 | <RL | 1.31 | 2.60 |
| Pv | M | A | 5.01 | 5.80 | 6.55 | 0.81 | 1.98 | 0.91 | 3.70 |
| Pv* | M | A | 12.75 | 16.45 | 34.45 | 3.30 | 2.95 | 6.44 | 12.69 |
| Pv | M | A | 4.58 | 6.13 | 15.56 | 2.34 | 1.40 | 4.07 | 7.81 |
| Pv | M | A | 3.71 | 5.51 | 6.98 | 1.38 | 0.62 | 3.48 | 5.48 |
| Pp | F | SA | 11.88 | 13.42 | 14.88 | 7.76 | 6.45 | 4.09 | 18.30 |
| Pp | F | SA | 8.14 | 9.58 | 11.32 | 6.92 | 7.22 | 3.72 | 17.86 |
| Pp* | F | A | 5.99 | 6.14 | 7.51 | 0.75 | 0.46 | 3.87 | 5.08 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Pp* | F | A | 13.52 | 15.01 | 15.90 | 5.61 | 7.02 | 11.99 | 24.62 |
| Pp | F | C | 10.75 | 15.35 | 16.61 | 3.53 | 3.58 | 5.21 | 12.32 |
| Pp | M | A | 0.55 | 0.72 | 0.95 | 0.11 | <RL | 0.59 | 0.70 |
| Pp | M | C | 0.63 | 0.79 | 0.94 | 0.07 | 0.26 | 0.63 | 0.96 |
| Pp | M | A | 0.50 | 0.69 | 0.77 | <RL | <RL | <RL | 0 |
| Pp | M | C | 1.44 | 1.91 | 1.94 | 0.69 | 0.40 | 2.28 | 3.37 |
| Pp | M | Y | 4.89 | 7.44 | 8.36 | 0.79 | 0.68 | 3.33 | 4.80 |

* = noticeable disease observed for this animal; M = male; F = female; A = adult; SA = subadult; Y = yearling; C = calf; NT = not taken; <RL = less than method reporting limit; DDTtot = ∑ p,p' DDT, p,p' DDD, p,p' DDE; PCB 1a = PCB congeners 77, 126, 169; PCB 1b = PCB congeners 105, 118, 128, 138, 156, 170; PCB 2 = PCB congeners 87, 99, 101, 153, 180, 183, 194

Four of the ill animals had total pesticide and PCB levels greater than 15 ug/g and 12 ug/g respectively (lipid weight basis). One of the sick animals had the highest total pesticide and PCB levels (45.11 and 24.86 ug/g respectively) of any of the animals examined (Table 2). Research on the effects of OCs on marine mammal health indicates that DDE and PCB concentrations similar to those found in our study can cause lowered testosterone levels in male Dall's porpoises (Subramaniam et al. 1987), increased risk of infectious disease in harbor porpoises (Hall et al. 2006), and suppressed immune function in harbor seals and harbor porpoises (de Swart et al. 1996, Beineke et al. 2005). However, further studies are needed in order to establish a clear cause and effect between contaminants and health in marine mammals.

DDE was the most prevalent organochlorine contaminant detected in blubber samples, with the average DDE content making up more than 58% of the total organochlorine pesticide concentration measured for each species (Figure 1). Mean DDE concentrations ranged from 4.39 ± 5.49 µg/g (lipid weight) for Steller sea lions (*Eumetopias jubatus*) to 9.79 ± 10.95 µg/g (lipid weight) for California sea lions (*Zalophus californianus*). Total PCBs ranged from 4.99 ± 8.59 µg/g (lipid weight) for Steller sea lions to 8.80 ± 8.81 µg/g (lipid weight) for harbor porpoises (*Phocoena phocoena*). There were no significant differences in the mean levels of contaminants between species (Figure 1), suggesting that there is negligible difference in risk of contaminant accumulation for consumption of a particular marine mammal species by avian scavengers. However, there was considerable individual variation in OC levels within species and across the data set (Table 2), ranging from a low of 0.20 µg/g DDE in an adult female Steller sea lion to a high of 34.37 µg/g DDE in an adult male California sea lion and a low of <10 µg/g $PCB_{tot}$ in several of the Steller sea lions and a harbor porpoise to 24.86 µg/g $PCB_{tot}$ in an adult male California sea lion. DDE levels (lipid weight) in blubber samples from the sperm whale, the Dall's porpoise and the elephant seal were 2.04, 1.06, and 3.78 µg/g respectively. Total PCBs for these species were 1.11, 1.23, and 0.44 µg/g respectively. Mean OC contaminant levels were higher in males versus females but this difference was not statistically significant likely due to the individual variability in OC levels in blubber samples. It will be important to take this individual variation into account when calculating potential long-term risk for avian scavengers. An earlier study of PCBs and DDE (based on wet weight) in harbor seals stranded on the Oregon coast also detected both OC contaminants, with DDE levels ranging from 0.4 to 12.5 µg/g, and total PCBs ranging from non-detectable to 6.1 µg/g wet weight (Hayteas and Duffield 1997). They also noted a high degree of intra-species variability. For example, the two female Steller sea lions evaluated in this earlier study varied from 1.7-31.3 µg/g, wet weight DDE, and from 1.1-8.7 µg/g, wet weight total PCBs, and 12 harbor porpoises varied from non-detectable levels to 9.4 µg/g, wet weight total DDT and 24 µg/g, wet weight total PCBs.

Figure 1

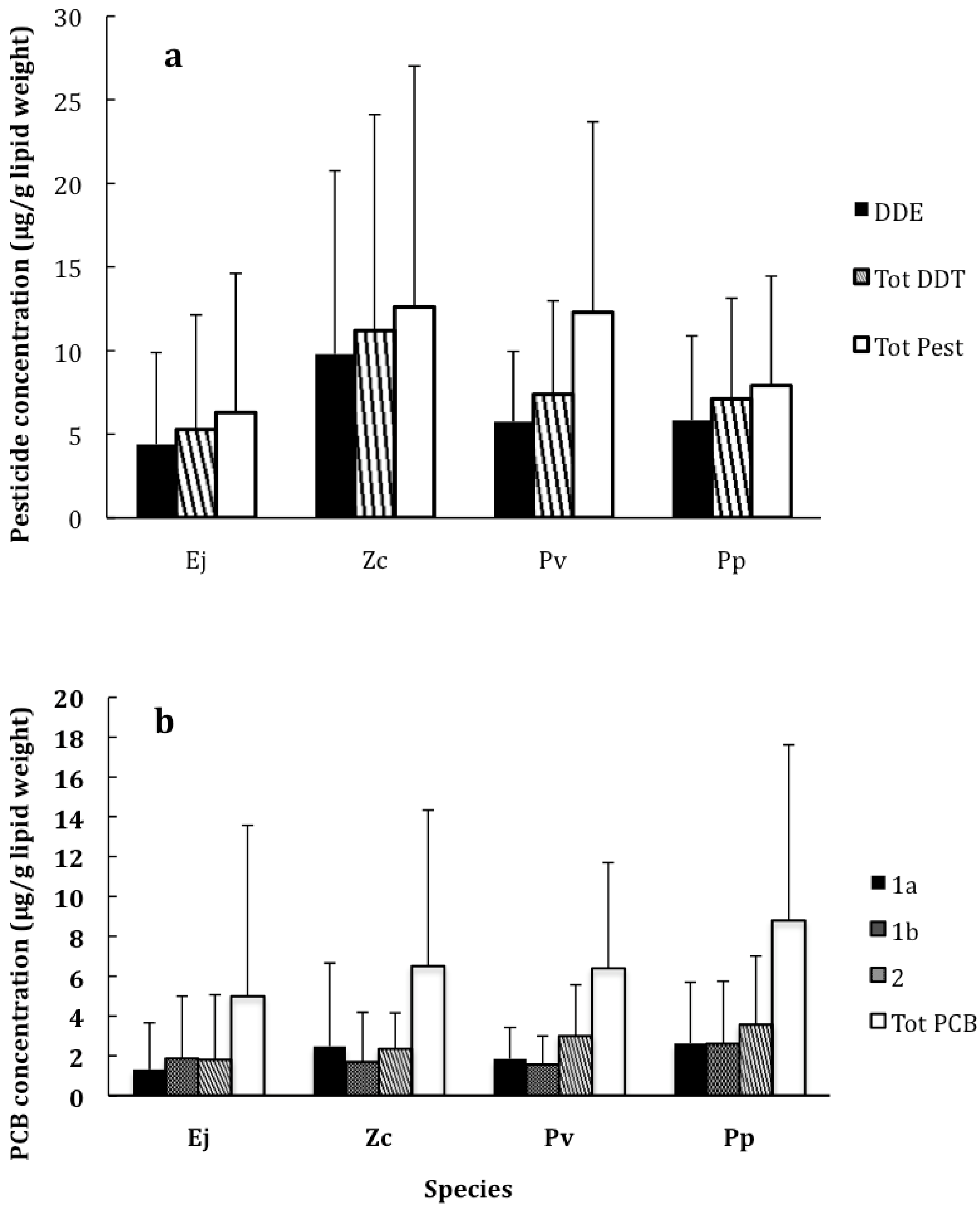
a
Pesticide concentration (µg/g lipid weight)
30
25
20
15
10
5
0
Ej
Zc
Pv
Pp
DDE
Tot DDT
Tot Pest
b
PCB concentration (µg/g lipid weight)
20
18
16
14
12
10
8
6
4
2
0
Ej
Zc
Pv
Pp
Species
1a
1b
2
Tot PCB

**Figure 1**. Mean (± standard deviation) organochlorine pesticide (A) and PCB (B) concentrations in blubber samples from Steller sea lions (Ej), California sea lions (Zc), harbor seals (Pv), and harbor porpoises (Pp). Tot DDT = DDT, DDD, and DDE; Tot Pest = total of all detected chlorinated pesticides; 1a = PCB congeners that are 3 methyl cholanthrene type inducers; 1b = PCB congeners that are mix type inducers; 2 = PCB congeners that are phenobarbitol type inducers.

Previous research on OC contaminants for pinnipeds along the California coast also indicated that DDE was the most prevalent contaminant in blubber and demonstrated intra-species individual variability from very low to very high levels (Blasius and Goodmanlowe 2008). All reported levels were substantially greater than those found in our study. For example, Blasius and Goodmanlowe (2008), found mean total DDT and PCB levels of 594 µg/g and 87 µg/g respectively (lipid weight) in blubber samples from California sea lions from the Southern California Bight. Harbor seals in the Southern California Bight had mean total DDT and PCB levels of 1041 µg/g and 123 µg/g respectively (lipid weight), The difference in contaminant levels between harbor seals and California sea lions was attributed in part to the fact that harbor seals tend to have localized areas of residence while California sea lion males move more widely through their range. Initial testing of condor eggs from nests in Central California raised concerns that the extremely high mean OC contaminant levels found in mammal carcasses from the California Bight may pose a serious threat to condor populations in this area (Walters 2008). Mean OC contaminants found in marine mammal blubber samples in our study are substantially lower than those found by the three California studies suggesting that condors released in Oregon are at lower risk for OC bioaccumulation than condors residing in Central California. However, the impact of accumulated contaminant loads in eagles nesting in the Lower Columbia River, Oregon has been shown to include significant thinning of eggshells and a drop in reproductive productivity to 30-50% that of eagles nesting in other parts of Oregon (Buck et al. 2005). Clearly further investigation of this issue is warranted to determine if bioaccumulation of these contaminants pose a risk to released condors in Oregon. In addition, evaluating whether the cumulative effects of both PCBs and DDE together would have a synergistically increased adverse effect on avian scavenger species feeding on beached carcasses is needed particularly when looking at immune suppression and endocrine disruption.

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