MEMORANDUM FOR: F/WRC/WCRO/PRD – Brent Norberg

FROM: F/NWC5 – Jennie Bolton

THROUGH: F/NWC5 – Gina Ylitalo

SUBJECT: Persistent organic pollutant and lipid analyses of blubber from a Southern Resident killer whale (Orcinus orca)

We have completed analyses for organochlorines, PBDEs (polybrominated diphenyl ethers) and lipids in a blubber sample collected from a juvenile female Southern Resident killer whale (L112) that was killed by blunt force trauma on or around February 2, 2012.

The report, “Persistent organic pollutant and lipid analyses of blubber of a deceased Southern Resident killer whale (Orcinus orca)” by Bolton is attached.

Please feel free to email or call Gina Ylitalo (gina.ylitalo@noaa.gov; 206-860-3325) if you have any questions about the analyses or data.

cc:
Gina Ylitalo – F/NWC5
Teri Rowles – F/PR2
Brad Hanson – F/NWC1
Walter Dickoff – F/NWC1
Persistent organic pollutant and lipid analyses of blubber of a deceased Southern Resident killer whale (*Orcinus orca*)

Jennie L. Bolton

**Introduction**

Persistent organic pollutants (POPs; e.g., DDTs, PCBs and chlordanes) are lipophilic compounds that have been used widely in the northern hemisphere in agricultural and industrial applications. Many of these compounds have been regulated because exposed wildlife can exhibit toxic effects, including immunosuppression and reproductive impairment (Ross *et al.*, 1995; Beckmen *et al.*, 2003; AMAP 2004). In addition, these contaminants are transported to the Arctic ecosystem via atmospheric processes, where they circulate and accumulate within the complex marine food web (Barrie *et al.*, 1992; Iwata *et al.*, 1993; AMAP 1998, Schmidt, 1998; de Wit *et al.*, 2004). POPs accumulate to high levels in tissues of marine predators, such as killer whales, due to biomagnification of POPs with increasing trophic level. Because “transient” killer whales feed primarily on other marine mammals (Ford *et al.*, 1998; Saulitis *et al.*, 2000), they are especially likely to accumulate high levels of POPs.

One group of POPs, the polybrominated diphenyl ethers (PBDEs), has elicited concern because of their recently reported wide geographic distribution in tissues of wildlife and humans (de Wit *et al.*, 2002). PBDEs are effective flame retardants, but are also highly persistent and bioaccumulative contaminants, with structures similar to the PCBs (AMAP 1998, Ikonomou *et al.*, 2002a,b, AMAP 2004). Exposure to PBDEs has been linked to various effects, including immune suppression, delays in reproductive development and impaired fetal brain development (Beineke *et al.*, 2005, Birnbaum and Staskal 2004). Furthermore, PBDE levels are increasing rapidly in marine mammals in the northern hemisphere (Ikonomou *et al.*, 2002b, LeBeuf *et al.*, 2004, Krahn *et al.*, 2009).

A sample of blubber from a juvenile female Southern Resident killer whale (L112) was analyzed for a suite of POPs (e.g., PCBs, DDTs and other pesticides, and PBDEs). The results of these analyses found that the tissues of this killer whale were moderately contaminated with these toxic chemicals.

**Analytical Methods**

*Sample collection*

This 3 year-old female Southern Resident killer whale (L112) from L Pod’s L4 matriline, was the second surviving calf of L86. The animal was found dead near Long Beach, Washington and the cause of death was determined to be blunt force trauma occurring on or around February 8, 2012. L112 was necropsied on February 12, 2012, and various tissue samples, including blubber, were collected, frozen and transported to NWFSC by Brad Hanson of NWFSC. They were transferred to the analytical lab for analysis on May 10, 2012.
**POP analyses by GC/MS**

Methods for POP analysis were described in Sloan et al. (2005). Briefly, blubber (a 0-2 cm depth from the skin was analyzed as this depth is most comparable to a biopsy sample) was extracted using accelerated solvent extraction (ASE) with methylene chloride. The sample extract was filtered through a column of silica gel and alumina and concentrated for further cleanup to remove interfering lipid compounds. This cleanup step used size exclusion chromatography with high-performance liquid chromatography (HPLC), which separated larger lipid molecules from the compounds of interest and allowed collection of the fraction containing the POPs. The HPLC fraction was analyzed for chlordanes, DDTs and other pesticides, PCBs and PBDEs by high resolution gas chromatography with low resolution mass spectrometry (GC/MS), with the mass spectrometer operated in selected ion monitoring (SIM) mode. Total PCBs ($\sum$PCBs) were calculated by summing the concentrations of 46 PCB congeners present as 40 chromatographic peaks (congeners 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208, 209). PCB and PBDE congeners are numbered according to the scheme in Ballschmiter et al. (1992). The total DDTs ($\sum$DDTs) were calculated by summing the concentrations of $o,p'$-DDD, $o,p'$-DDE, $o,p'$-DDT, $p,p'$-DDD, $p,p'$-DDE, and $p,p'$-DDT; $\sum$chlordanes is the sum of oxychlordane, gamma-chlordane, nona-III-chlordane, alpha-chlordane, trans-nonachlor, and cis-nonachlor; $\sum$HCHs (hexachlorocyclohexanes) is the sum of alpha-, beta-, and gamma-HCH isomers and $\sum$PBDEs is the sum of congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 155, and 183, plus 2 pentabrominated, 1 hexabrominated, and 1 heptabrominated congeners whose congener numbers are not known.

**Lipid Determination by TLC/FID**

Blubber of L112 was analyzed for lipid classes and concentrations by TLC/FID using an Iatroscan Mark 5 (Iatron Laboratories, Tokyo, Japan) as described by Ylitalo et al., (2005). Five classes of lipids (i.e., wax esters, triglycerides, free fatty acids, cholesterol and polar lipids) were separated based on polarity. The total lipid (total extractable organics) reported was determined gravimetrically.

**Results and Discussion**

The relative percentages of five lipid classes in blubber are shown in Table 1. The sample had no free fatty acids present, which indicates that the blubber sample was not subject to decomposition prior to analysis.

Concentrations of POPs are shown in Table 2. Overall, ranked concentrations were $\sum$DDTs > $\sum$PCBs >> $\sum$ chlordanes > $\sum$PBDEs > HCB > $\sum$HCHs. On a lipid basis, concentrations of HCB in the blubber of L112 (870 ng/g lw) were substantially higher than those in two somewhat older (15 year-old) juvenile males from L Pod (L78, Krahn et al. 2007, 600 ng/g lw; L87, Krahn et al. 2009, 350 ng/g lw). Concentrations of PCBs in the blubber of L112 (27,000 ng/g lw) were comparable to but somewhat higher than those animals (L78, Krahn et al. 2007, 22,000 ng/g lw; L87, Krahn et al. 2009, 24,000 ng/g lw). PCB concentrations were somewhat lower than the mean of $\sum$PCBs in biopsy
samples collected between 1993 and 1996 from adult male northern resident killer whales, as reported by Ross *et al.* (2000) (a mean of 37,400 ng/g lw, n=8).

Total DDTs measured in the 0-2 cm blubber layer of L112 (Table 2) were ~2 times higher than those measured in the same layer of an adult (30 year-old) female southern resident killer whale, L60, that stranded in Washington state in 2002 (Krahn *et al.* 2004) (43,000 ng/g lw vs. 19,400 ng/g lw, the average of five locations). The $\Sigma$DDT/$\Sigma$PCB ratio in blubber from L112 (~1.6) was somewhat higher than the ratio of 1.1 determined from the mean of the $\Sigma$DDT and $\Sigma$PCB concentrations from the 0-2 cm layers from five locations on L60 reported in Krahn *et al.* (2004). Concentrations of DDTs were very similar to those in two somewhat older (15 year-old) juvenile males from L Pod (L78, Krahn *et al.* 2007, 38,000 ng/g lw; L87, Krahn *et al.* 2009, 44,000 ng/g lw).

Total PBDEs measured in the 0-2 cm blubber layer (most similar to a biopsy sample) of L112 (Table 2) were approximately sixteen times higher on a lipid basis than PBDE concentrations measured in biopsies of male northern residents reported by Rayne *et al.* (2004) (3,300 ng/g lw vs. a mean of 203 ng/g lw, n=9). Concentrations of PBDEs were comparable to but somewhat higher than those in two somewhat older (15 year-old) juvenile males from L Pod (L78, Krahn *et al.* 2007, 2,600 ng/g lw; L87, Krahn *et al.* 2009, 2,600 ng/g lw).

Although PBDEs are an emerging concern in marine and terrestrial biota, few recent measurements have been made in killer whales or other species. Most published measurements have been made on archived samples, as was true of the samples reported in Rayne *et al.* (2004), which were collected between 1993 and 1996. Because PBDEs are still used in North America, environmental PBDE levels may continue to rise. Due to this and biological factors such as maternal offloading of contaminants during gestation and lactation, juvenile killer whales may be particularly at risk, and higher average levels of PBDEs compared to adults have been measured recently in juvenile Southern Resident killer whales (Krahn *et al.* 2009), as well as insular Hawaiian Island false killer whales (Ylitalo *et al.* 2009).

**References**


Beckmen KB, Blake JE, Ylitalo GM, Scott JL, O'Hara TM (2003) Organochlorine contaminant exposure and associations with hematological and humoral immune


In: G.K. Ostrander (Ed.), *Techniques in Aquatic Toxicology-Volume 2*. CRC Press, Boca Raton, FL.

Table 1. Lipid classes* in blubber of a juvenile female Southern Resident killer whale (L112) stranded near Long Beach, Washington, in February 2012.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>SALE (% of Total)</th>
<th>TG (% of Total)</th>
<th>FFA (% of Total)</th>
<th>CHOL (% of Total)</th>
<th>PL (% of Total)</th>
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<tr>
<td>0-2</td>
<td>19.8</td>
<td>80.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Lipid classes are measured by TLC-FID to precision of 0.1%.
SALE = stearic acid laurel (wax) esters; TG = triacylglycerols; FFA = free fatty acids; CHOL = cholesterol; PL = phospholipids

Table 2. Concentrations (ng/g, wet wt or ng/g, lipid) of POPs in blubber of a juvenile female Southern Resident killer whale (L112) stranded near Long Beach, Washington, in February 2012.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Lipid %*</th>
<th>HCB</th>
<th>∑HCHs</th>
<th>∑CHLDs</th>
<th>∑DDTs</th>
<th>∑PCBs</th>
<th>∑PBDEs</th>
<th>HCB</th>
<th>∑HCHs</th>
<th>∑CHLDs</th>
<th>∑DDTs</th>
<th>∑PCBs</th>
<th>∑PBDEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>72.8</td>
<td>630</td>
<td>390</td>
<td>4,100</td>
<td>31,000</td>
<td>20,000</td>
<td>2,400</td>
<td>870</td>
<td>530</td>
<td>5,600</td>
<td>43,000</td>
<td>27,000</td>
<td>3,300</td>
</tr>
</tbody>
</table>

* Lipid % was measured gravimetrically as total extractable organics; POPs concentrations are reported to two significant figures.