Using Blood Samples to Monitor Chlorinated Hydrocarbon Exposure of Bald Eagles on the Northern Channel Islands of California

A Report to the Science Review Panel for the Northern Channel Islands Bald Eagle Feasibility Study

John E. Elliott
Canadian Wildlife Service
5421 Robertson Rd
Delta, BC, Canada
V4K 3N2

Background

The U.S. Fish & Wildlife Service proposes to release a limited number of bald eagles onto Santa Cruz Island as a feasibility study to reintroduce the species into former habitat on the Northern Channel Islands of California. Further details, a rationale and risk assessment are described in Valoppi *et al* (2000). The risk assessment raises a number of questions regarding the likelihood of success of this release due to ongoing contamination of regional food chains by DDE.

This report considers the potential for assessment and continued monitoring of released bald eagles for chlorinated hydrocarbon exposure, particularly DDE, via trapping and blood sampling of released birds.

Introduction

Use of blood samples as a non-destructive technique for assessing both exposure and effects of environmental toxicants in avian wildlife has become increasingly common in recent years. Advances in analytical methodology now allow for routine measurement of a variety of chlorinated hydrocarbon and trace metal contaminants in blood samples, while development of biomarker techniques permits assessment of a variety of toxicological endpoints.

Examples of effective use of blood sampling in avian contaminants investigations include monitoring of organochlorine levels in migrating raptors (Henny et al, 1988, Elliott & Shutt 1993) and nestling bald eagles (Bowerman 1993, Dykstra et al 1998, Elliott & Norstrom 1998, Matz 1998, Welch 1994).

Relationship between contaminant levels in blood and body burden

Compounds, such as DDE and many PCB congeners are highly lipid soluble and are thus stored primarily in body fat. They are also very slowly metabolized with half-lives in the order of one year in birds. Because blood perfusion rates of lipid storage tissues, such as fat depots, liver and muscle are very high and rates of metabolism are low, persistent OCs exhibit relatively constant equilibrium between concentrations in blood and in other tissues. As a result, blood sampling is considered a valid method for measuring body burden of persistent organochlorine contaminants. Studies in humans and various wildlife species have shown consistent relationships between levels of OCs in blood and other tissues (Mes 1992, Bernhoft et al 1997, Friend et al 1979, Bustnes et al 2001).

Many birds undergo large seasonal changes in body fat stores caused by stresses of reproduction and varying food availability. Because of the relatively rapid equilibration process between plasma and lipid stores, changes in body weight and condition and associated mobilization of fat stores and dissolved OCs should be evident in plasma concentrations. Studies of breeding birds of different species have shown such trends (Henriksen et al 1996, van den Brink et al 1998). Despite the potential for the effect of changes in body condition,

plasma concentrations of OCs in blood of individual incubating adult glaucous gulls remained consistent between years (Bustnes et al 2001).

Nestling bald eagles sampled from a variety of sites on the coast of British Columbia (B.C.) had significant differences in mean lipid content of plasma samples among sites, and there was a significant positive relationship between mean plasma lipid content of nestlings and mean productivity (Elliott & Norstrom 1998, Elliott et al 1998). The differences among sites in average lipid content of plasma were thought to be caused by variation in body condition of nestlings related to food delivery and therefore of local prey availability. In that case, comparison of contaminant levels among sites was normalized by using analysis of covariance to adjust for differences in lipid content.

Most studies of contaminant concentrations in wildlife blood have not reported or discussed the influence of food intake and fasting on plasma lipid concentrations. In a study of humans, Phillips et al (1989) found that concentrations of OCs in serum can be affected by fasting and feeding. For birds, there are no similar laboratory studies which report variation in plasma lipid and contaminant concentrations related to feeding. In chickens, serum lipid concentrations increase rapidly during meal periods, but return to steady-state concentrations within about 2 hr after feeding (LeClercq et al 1974). In the studies of Elliott and co-workers cited above (Elliott & Norstrom 1998, Elliott et al 1998), they measured lipid content of bald eagle nestling plasma using traditional hexane-extraction gravimetric techniques to determine total lipids. In a sample of 52 nestling bald eagles from a wide area of the B.C. coast, they found a 40-fold

variation in plasma lipid content among individual birds. Samples with lipid content greater than 2 standard deviations outside of the mean were thought to have been collected soon after feeding.

The influence of the post-feeding flux of nutrients and lipids on plasma lipid and contaminant levels can be avoided by attempting to trap birds during times of the day when they are not normally feeding. Highly lipemic samples can be screened out by visual inspection, as the blood sample will appear cloudy and a lipid plug will be evident after centrifugation. Potentially, eagles could be kept for an additional 2 hr and sampled again before releasing; however, that could cause undue stress to the bird and is not recommended. All samples should also be measured for plasma lipid content using standard techniques (e.g. Mes 1987, Drouillard et al 1999). Samples with lipid content outside a normal range can be screened out or adjusted for lipid content using statistical methods (Hebert & Keenleyside 1995, Elliott & Norstrom 1998).

Effect of age on contaminant body burden

Bald eagles will be hacked out on Santa Cruz Island as nestlings. What age should trapping of those birds first be attempted? Herring gulls fed a diet of highly contaminated Great Lakes fish took up to 2 years for body burdens of DDE and PCBs to equilibrate with dietary concentrations (Anderson & Hickey 1976). Given the high concentrations of DDE in the prey species of the Channel Islands, the larger body size and expected low clearance capacity of bald eagles, equilibration could take an even longer period. Attempts to trap the released birds should not begin until they are at least one year of age, preferably older.

Consideration should be given to a strategy of placing both a solar powered satellite transmitter and a conventional transmitter with long battery life on all released birds. This should provide 3 years or more of tracking capability. Detailed data on habitat use will permit identification of feeding and roosting sites for individual eagles, which should be valuable for both diet studies and trapping efforts.

Interpretation of plasma contaminant residues in juvenile and sub-adult eagles

Trapping of released birds is technically feasible, and we can assume that plasma concentrations of OCs will be representative of body burden. However, there are currently no published criteria for DDE or other OCs in plasma of sub-adult or adult eagles for assessment of reproductive effects. Concentrations of DDE in nestling plasma ranging from 27.8 to 40.9 μg/kg associated with 0.7 young/occupied territory have been tentatively suggested (Elliott & Harris, in press). However, those numbers can be considered to be of little value in interpreting plasma concentrations of breeding adults or older juveniles, which can be expected to be much higher in a given environment.

There are very limited reported data on plasma concentrations of juvenile or adult bald eagles with which to conduct comparisons. Available published and some unpublished data are shown in Table 1. With the exception of the DDE-poisoned bird from Catalina Island, concentrations in adults range from 0.06 μg/g from 4 birds sampled in 1978 in Missouri to 0.77 μg/g in 4 birds trapped in 1997 on the B.C. coast. In birds classified as sub-adults, concentrations were 0.06 to 0.07 μg/g plasma. Given that those samples from sub-adults were collected from

areas without substantial local DDE contamination problems, they may provide a useful reference for interpreting data from the Channel Islands.

In order to provide a tentative reference value, or range of values, for sub-adult eagles, I would suggest that statistical analysis of any existing plasma samples from Catalina Island be undertaken, and hopefully more sub-adults and adults from that area of known DDE contamination be trapped and sampled. A reference population should also be identified, and simultaneous program of trapping and sampling of sub-adult and adult eagles should be implemented at that site. An ideal reference population would be located in a marine environment along a neighboring area of the coast with similar diet and which was less impacted by DDE contamination. However, given the lack of such a population, the eagles nesting on reservoirs in southern California likely furnishes the best reference group. Recent comparative data on both contaminants and productivity are already available for marine and estuarine eagles from further north along the Pacific coast in Oregon, Washington and British Columbia.

Consideration also should be given to cooperating with other agencies who may already have archived samples of sub-adult or juvenile bald eagles, particularly in areas with a gradient of DDE contamination and nesting success.

<u>Measurement of other contaminants in blood</u>

Although the primary concern with the Channel Island eagles is DDE contamination, consideration should be given to screening blood samples for other environmental toxicants. The chemicals discussed below can all be

assayed in blood using standardized and inexpensive techniques which require relatively small volumes of sample (about 0.5 ml maximum per assay).

There is recent concern over global increases in mercury contamination. Published data on critical concentrations in avian blood are available (Evers et al. 1998. Because of their tendency to scavenge on bird and mammal carcasses, bald eagles are also at risk of exposure to a variety of toxicants, including lead, anti-cholinesterase insecticides, and rodenticides. Lead poisoning of bald eagles is a well known issue. Critical concentrations of lead in avian blood are well known (Pain 1996). Although the potential for exposure to anticholinesterase compounds to Channel Islands bald eagles is low, significant depression of plasma cholinesterase activity can persist for many days following exposure to an organophosphorus compound (Elliott et al, 1997). The normal range of plasma cholinesterase is well established for bald eagles. If brodifacoum or other persistent anti-coagulant rodenticides are being used for rat removal on neighboring islands, blood samples can also be screened for evidence of exposure to those compounds (Howald et al 1999).

Telemetry data could be used to determine the potential need for screening for other contaminants.

Measurement of bio-markers in blood

DDE impacts bird populations primarily by affecting shell quality and therefore hatching success. Thus, the primary need is to establish a critical concentration that can be expected to be associated with reduced nesting success, as

discussed above. However, exposure to elevated concentrations of DDE and other DDT-related compounds may affect a variety of sub-lethal physiological functions.

As a minimum, haematocrits should be determined on all samples and blood smear prepared in order to determine white blood cell counts, a simple but gross assay for possible immune system function. The presence of blood parasites can also be assessed from a blood smear.

The effects of OCs on concentrations of retinoid (vitamin A) and thyroid compounds in blood has been undertaken in a number of studies of birds (e.g. Murvoll et al 1999, Elliott et al 2001). Plasma transport of both retinol and thyroxine is via a common protein complex, and can be affected in bald eagles by hydroxylated PCB metabolites (Newson 2000). Given there is some concern about ongoing PCB contamination in the California bight, consideration should be given to measurement of plasma retinol and T4.

Recommendations

1. All released eagles be colour banded and tagged with satellite and conventional radio telemetry transmitters. The transmitters would have to be attached prior to fledging, but after birds have completed their growth.

2. Movements of birds be tracked to determine when and where they eventually establish some residency on the island(s). Feeding areas can also be located, prey observations made and pre-baiting conducted.

3. Trap juvenile eagles within a minimum of 1 year of release. It may be preferable to wait until birds are 2 to 3 years of age.

4. Identify and establish a suitable reference area. Trap and blood sample juvenile and adult eagles at that site.

5. Blood sample as many Channel Islands eagles as possible. Attempt to develop a value or range of values for DDE in blood samples from the Catalina Island population as tentative criteria for indication of significant effects on reproduction.

6. Analyze any existing samples.

7. Develop a protocol designed to optimize use of blood samples for the following assays (listed in approximate descending order of suggested priority):

1. OC/PCB compounds in plasma.

2. Total mercury in whole blood.

3. Total lead in whole blood.

4. Haematocrit and blood smears for cell counts and parasites.

5. Standard veterinary blood screening.

6. Rodenticide residues in plasma.

7. Cholinesterase activity in plasma.

8. Vitamin A and thyroid hormones in plasma.

NOTES: 1) In addition, small quantities of blood and/or plasma may need to be retained for stable isotope studies.

2) Any remaining plasma should be archived at - 20^0 C or colder.

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Table 1
Chlorinated hydrocarbon concentrations (mg/kg wet weight) in blood/plasma of adult and juvenile bald eagles, expressed as geometric means, 95% confidence limits (in brackets) and ranges[a]

Region	Location	Years	Age[b]	Tissue	N	p,p'-DDE	Dieldrin	Total PCBs	Source[c]
	Vancouver	1997	A	plasma	3	0.09 0.05 - 0.19	<0.001	0.09 0.06 - 0.21	#
	Barkley Sound	1997	A	plasma	3	0.20 0.19 - 0.21	0.003 0.002 - 0.005	0.40 0.33 - 0.53	#
	Crofton (north)	1997	A	plasma	3	0.77 0.43 - 1.53	0.008 0.006 - 0.01	0.95 0.58 - 2.19	#
	Crofton (south)	1997	A	plasma	4	0.28 0.13 - 0.56	0.002 <0.001 - 0.006	0.66 0.21 - 1.29	#
California	Santa Catalina Island	1993	A	serum	1	53	-	26.0	1
Colorado[d]	San Luis Valley	1977	A	plasma	10	0.14 0.03 - 0.23	0.01 <0.01 - 0.05	0.1 <0.01 - 0.68	2
			SA	plasma	10	0.07 <0.01 - 0.31	<0.01	0.04 <0.01 - 0.36	2
Missouri[d]	Swan Lake National Wildlife Refuge	1978	A	plasma	4	0.06 0.03 - 0.10	0.03 nd - 0.08	0.24 nd - 0.36	2
			SA	plasma	11	0.06 0.01 - 0.14	0.01 nd - 0.06	0.08 nd - 0.27	2
				blood	16	0.04 (0.02, 0.06)	-	0.02 (0.01, 0.04)	3
				blood	5	0.03 (0.01, 0.13)	-	<0.05 <0.05 - 0.08	3

[a] '-' = data not given; 'nd' = not detected, but limit of detection not provided
[b] A = adult, SA = subadult, J = juvenile, N = nestling
[c] # - CWS, unpubl, data.
1 - Garcelon & Thomas, 1997
2 - Henny et al, 1991
3 - Frenzel & Anthony, 1989