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Department of Fish and Game
Habitat Conservation Planning Branch

Final Report Addendum:

ANALYSIS OF LEAD IN CALIFORNIA CONDOR FEATHERS: DETERMINATION OF EXPOSURE AND DEPURATION DURING FEATHER GROWTH

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Introduction

This report is an addendum to the report: "Assessment of Lead Contamination Sources Exposing California Condors", Final Report, submitted to the Department on April 7, 2003. The report covers the additional work agreed to in the revised statement of work of October 3, 2002, and reflects work conducted between October 15, 2002 and April 30, 2003.

The objectives of the contact extension were to undertake the following tasks:

1. Determine, in collaboration with Prof. Don Smith and graduate student Molly Church at UC Santa Cruz, the feasibility of using condor feathers to monitor the time-course of lead exposure:

2. Determine the sensitivity of methods for lead analysis of feathers, and determine the sample mass necessary for baseline measurements of lead in condor feathers using inductive coupled plasma mass spectroscopy.

3. Determine the time resolution that can be obtained in the separation of sequential samples from feathers, based on: Snyder, Johnson and Clendenen, 1987, "Primary Molt of California Condors" *The Condor* 89:468-485

4. Measure lead residues in condor feathers and archived samples of liver and bone from a sample of birds that were diagnosed to have died from lead intoxication. Obtain lead residue values from feathers that were growing at the time of death, and correlate feather lead levels with blood, liver, kidney, and bone lead levels that were taken at necropsy.

5. Estimate from feather residues the time-course of lead exposure of condors known to have been exposed to lead by sequential sampling and analysis of feather shaft protein along the axis of the feather.

Each of the tasks was initiated, although successful completion of some tasks was impossible, because of logistic complications arising from collection and delivery of condor feather samples to UC Davis for analysis. The complications will be explained in subsequent sections. Part of objective 4 is being investigated as part of the thesis topic of Molly Church's graduate work in environmental toxicology at UC Santa Cruz. As a result, samples of liver and bone were not evaluated in this study.

Specific Work Plan

The work for this extension was separated into 4 sections:

A. Description of feather growth, and analysis of condor primary and secondary feather growth to determine the possibility of lead analysis sequentially along a single feather.

B. Evaluation of available lead assay methods, and determination of the potential sensitivity of lead analysis of single feathers.

C. Analysis of molted condor feathers, to determine lead exposure in the field.

D. Extrapolation of lead analysis data for future work.

Goals Achieved

A. Description of feather growth, and analysis of condor wing feather growth to determine the possibility of lead analysis sequentially along a single feather.

Feathers are inert epidermal structures composed primarily of the protein keratin, which grow from specific epidermal follicles arranged in a complex pattern in feather tracts over the body of a bird (Romanoff, 1955). There are a very defined number and pattern of feather follicles, and the feather that grows from each follicle is unique in color, shape, size and pattern. Feathers do not grow continuously, like hair, nails, or horns, but grow rapidly to their final size, and remain attached to the quiescent follicle as an epidermal appendage throughout the life of the feather. Feathers are periodically replaced in a specific molt pattern, with feathers of some species replaced twice a year, some only once per year. Flight feathers of very large birds are generally replaced only once every two years. Bird species with alternate breeding and non-breeding plumages replace most or all of their feathers twice a year, with each feather alternatively growing out in the breeding plumage pattern, or in the alternate plumage. Other species of birds, including condors, do not have specific breeding plumages, but feather color and morphology usually differ between juvenile and adults, with the adult plumage replacing the juvenile feathers in a progressive molt. The flight feathers of condors, including the wing primaries and secondaries, and the tail feathers are replaced on a sequential two-year cycle (Snyder et al 1987).

Feathers grow from epidermal follicles with the distal tip of the feather emerging first from the follicle, and drying into the final configuration as more proximal portions are continuously added. The blood feather in Figure 1 demonstrates the appearance of a growing feather.

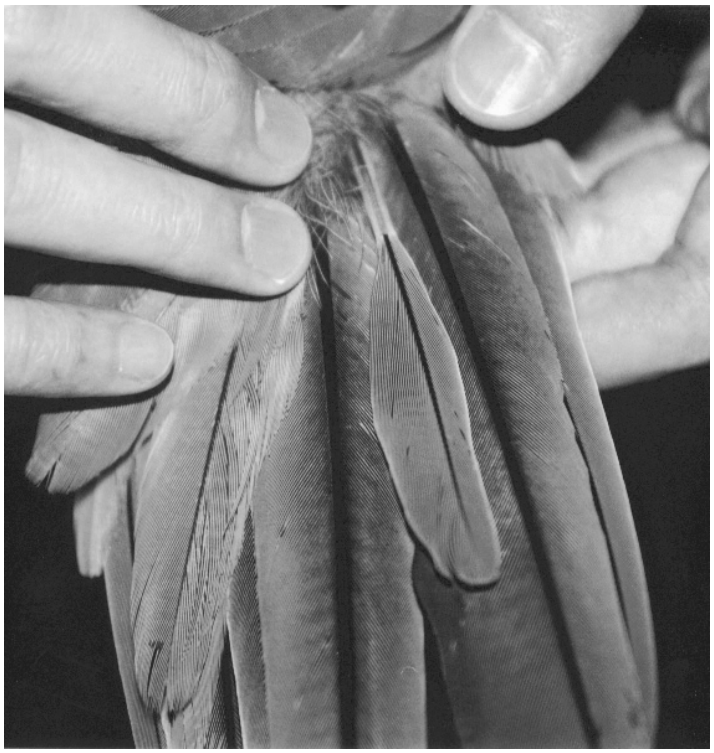


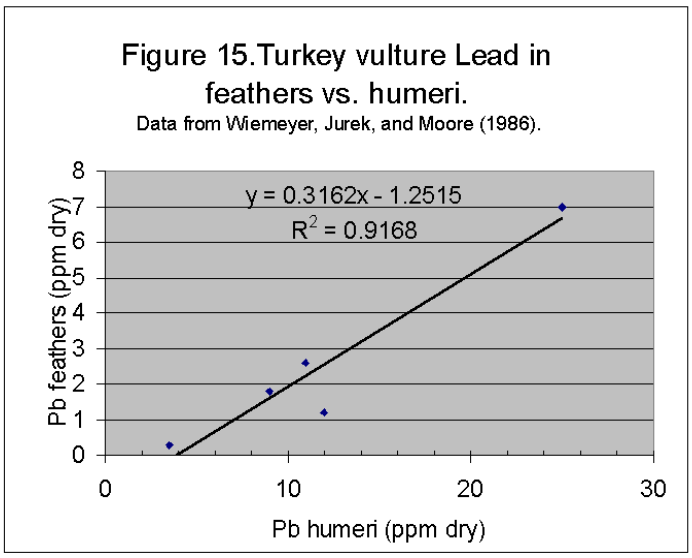
Figure 1: Blood feather growing from the tail of a captive macaw. The shaft of the growing feather is soft and pulpy where it emerges from the follicle, and hardens as the feather emerges. The vane emerges from the follicle in a tubular shaft, which splits, allowing the feather to unroll from its tubular growth configuration. The growth of a single feather may take up to 4 months in condors.

Metals accumulate in feathers by active or passive diffusion from the blood into the feather follicle, and deposition into the protein matrix at the time of growth. Metal concentrations in the feather growth region are thought to be proportional to circulating levels of metals in the blood. Because mature feathers lose most of the moisture present in the growing tissue, the concentration of metals in feathers do not equal the blood levels, but are usually considerably higher. Any metal incorporated into a feather during growth will remain in the portion of the feather where it was deposited. Analysis of portions of a feather along its length should provide a record of the presence of the metal in the circulation of the bird at the time of feather growth.

Previous studies have demonstrated highly elevated lead concentrations in feathers of vultures and condors, indicating that lead exposure of a condor during feather growth will result in lead incorporation into the feather structure. During the early period of the Condor Recovery Program, several studies were conducted to determine the extent of lead in the condor range, by measuring lead in food items, condor feathers, and in golden eagles, ravens and turkey vultures throughout the range. Wiemeyer et al (1983), Wiemeyer et al (1986), and Pattee et al. (1990), all presented data on biological tissues collected in California. The levels in eagles, ravens and vultures were similar to the levels found in condors at capture or necropsy (see final condor report for data, Tables 5 and 7), reflecting wide spread bio-available lead in the condor range. Whole condor feathers contained up to 14 ppm lead, reflecting exposure during the period of feather growth (Wiemeyer et al 1986).

The quantitative relationships between lead residues in feathers and residues in other tissues must be extrapolated, because no data has been collected simultaneously from tissues to correlate lead in blood, liver, bone and feathers. "Ball-park estimates" can be made, however, for the purpose of estimating whether feather lead analysis might be sensitive enough to evaluate lead exposure during feather growth. Wiemeyer et al (1986) presented data on liver, bone, and feathers, which have been graphed below to evaluate correlations between the tissues. The study found better correlation between levels in feathers and bone than with feathers and liver or bone and liver.

Figure 2 (Figure 15 taken from the Final Report) gives data plotted from their study, in which all the secondary feathers from one wing were ground homogeneously and levels compared to bone samples taken from the femur. Since the secondary molt in vultures occurs on a gradual two-year cycle, homogenizing all of the feathers from one wing essentially averages the lead exposure during that period. This long-term integration can be thought of as analogous to the slow turnover of bone (T ½ >600 days), and the residue data from these two tissues correlate well (R^2=0.917), although bone accumulates about 3 times as much lead as feathers on a dry weight basis.



Blood, liver, kidney, bone and feathers have all been used as tissues for reference levels of lead in birds, including condors. The levels of lead in each tissue vary greatly, and the half-time residence life (T ½) in each tissue is different as cited in Section II. E. of the Final Report. Comparisons of tissue residues within a single individual reveal that there may be no close time correlation between lead levels in different tissues at one point in time. For example, if lead has a T ½ of 14 days in blood and 600 days in bone, a short-term acute exposure to lead may show a significant spike in blood levels, but only a small fraction of the bone will pick up any lead. Conversely, chronic low-level lead exposure may result in substantial accumulation in bone with only low concentrations in blood. The data for condors "Broken Feather" and "Tehachapi" (Final Report Table 2, Table 7) are good examples of the lack of correlation in lead levels between tissues. "Broken Feather" liver lead was 23 ppm, and bone was 72 ppm, while "Tehachapi" liver level was 1.6 ppm, and bone was 79 ppm. The liver residues of 1.6 ppm would be considered only marginally elevated, but the 79 ppm in the bone indicates significant long-term lead exposure.

Correlations between liver lead levels at necropsy and blood levels taken just before death may also not correlate well. Scott and Jurek (1985) documented condors with blood levels up to 1.2 ppm lead for birds that survived, and liver lead levels of 23 and 35 ppm for condors that had died. The data of Pattee et al (1981) with bald eagles indicated that birds could die with as little as 4 ppm blood lead, and some birds survived with blood levels of 8-10 ppm. Because liver probably accumulates lead during an acute exposure, liver levels are likely to continuously increase above blood levels. Although the correlations are weak, the data indicate that feathers and bone accumulate more lead than is detected in blood, and the levels in a feather should be detectable on a daily or short-term duration during feather growth. It is probable that feather levels will be higher than blood levels, because of the desiccation of the feather as it matures, with the feather level several times the blood level.

Snyder et al. (1987) analyzed primary molt and feather growth in California Condors, and determined that growth of a primary feather takes approximately 105-120 days (3 ½-4 months), with a growth rate of about 5mm/ day.

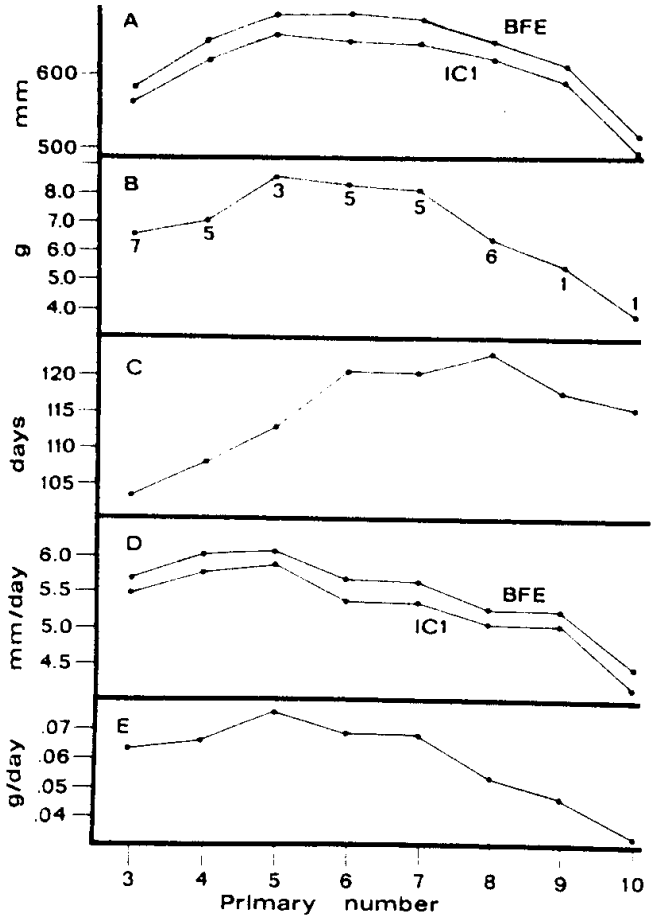


Figure 3 is a copy of Snyder et al., 1987 Figure 6, showing the differential size and growth rates of numbered condor primaries. The average lengths of primaries from two birds is given in graph A, the weights of individual primary feathers in B, and the average time for feather replacement, derived from photographic analysis of wild condors is given in C. The calculated rate of growth in mm/day is given in D and the growth in g/day is given in E. These data are extremely useful for making estimates of the amount of feather needed for analysis, and the time resolution that might be obtained if feathers were cut into sections for analysis.

Figure 3. Growth of California Condor Wing Feathers. From Snyder et al. 1987.

Condor flight feathers are large, weighing 4-8 g, which would provide a relatively large amount of material to analyze. The amount of feather material added each day is approximately 4.5 to 6 mm, with a weight of approximately 0.04 to 0.07g. This information gives a good estimate with which to evaluate the resolution that might be obtained with sequential feather segment analysis.

B. Evaluation of available lead assay methods, and determination of the potential sensitivity of lead analysis of single feathers.

Commercial analytical laboratories normally analyze lead by either of two methods: Graphite furnace atomic absorption spectroscopy (GFAAS), or inductive coupled plasma (ICP). Initial studies for this report were conducted at the UC Davis Center for Animal Health and Food Safety (CAHFS) Toxicology Section using ICP, and comparing with GFAAS.

The much more sensitive technique of ICP mass spectroscopy (ICP-MS) is a research technique, with very limited availability. The University of California at Santa Cruz Department of Environmental Toxicology, and the UC Davis Center for Mass Spectroscopy of Trace Metals both have instruments capable of quantifying lead and lead isotopes at very high resolution.

The minimum detection limit (MDL) for ICP at CAHFS is approximately 0.1ppm, using a sample size of 0.5g (CAHFS Method 8071). ICP has the capacity to analyze multiple metals from a single sample, but at increased cost, and only lead was analyzed in the initial screening test. GFAAS has a somewhat lower MDL for lead, of about 0.06 ppm in tissue (0.1g sample) or blood (sample volume of 50ul), but is capable of analysis of only a single metal species for each sample (CAHFS Method 8083).

ICP MS is capable of much lower detection limits, being able to quantify 10 ppb (0.01 ppm) with a 50 mg tissue sample (Don Smith, UC Santa Cruz, personal Communication). ICP MS is more versatile, in that it is also able to determine the lead isotope ratios in samples, so that identification of lead sources is a distinct possibility. ICP MS should be able to differentiate atmospheric lead background from a specific metallic lead ingestion event, and be able to compare the isotope ratios of any recovered lead fragment with the lead isotope pattern of lead incorporated into the growing feather follicle.

Each of the above techniques has specific advantages. ICP and GFAAS are relatively inexpensive, at a cost of about $4.00 per sample for a measurement of lead only. At present, ICP-MS is only available through cooperative research agreements with the Univ. of California at Davis or Santa Cruz, but was not available at UC Davis at the time of this study.

The sensitivity of lead analysis has increased greatly since the work of Wiemeyer et al (1986). Either of the commercial techniques currently available (GFAAS or ICP) is capable of measuring lead in portions of a single condor flight feather. The minimum detection limit is the limiting factor for the sample size, and since the correlation between blood and feather lead levels can only be estimated, the sample size can only be derived empirically.

From the discussion on feather growth above, feathers could be expected to incorporate lead directly from blood, and to concentrate it somewhat as the feather matures and dries.

If the MDL is desired to be in the same range as the clinical monitoring for lead (10-100 μg/dl, equivalent to 0.1-1 ppm), samples of 0.1 g could be analyzed by GFAAS, and samples of 0.5 g could be analyzed by ICP. Samples as small as 5 mg could be analyzed by ICP-MS to give equivalent sensitivity.

Snyder et al (1987) calculated growth of approximately 0.04-0.06 g/ day as the weight of material incorporated into condor primary feathers. Using this amount of material, the time resolution obtainable with ICP would be an increment of about 10 days, for GFAAS about 2 days, and daily increments of feather growth could be analyzed by ICP-MS. If feathers concentrate lead to greater levels than blood, the time resolution for each of the techniques would be improved.

C. Analysis of molted condor feathers, to determine lead exposure in the field.

I made requests for two sets of condor feathers for lead analysis in this study. A study protocol for analysis of feathers from dead condors stored by the USFWS in freezers at Ventura and at the San Diego Zoo was submitted in September 2002, outlining the request for collection of feathers that were growing at the time of death. Bruce Palmer and Bob Riseborough collected feathers from condor carcasses in October 2002. The feathers were delivered to Dr. Chamberlain at Stanford, and to D. Smith at UC Santa Cruz, but none were delivered to me for this study. I obtained a single feather from Condor 191 in March, 3002, just before the closure of this study, but that feather was not analyzed for reasons to be discussed below.

I made an additional request for molted feathers to the Condor Program at Hopper Mountain in December 2002, to evaluate the potential for detecting lead exposure in molted feathers. Twenty-six California Condor primary and secondary feathers and one white under-wing covert feather were supplied by the team at Hopper Ranch. The feathers were molted feathers retrieved from the condor flight pens after birds had been captured from the field and subsequently released. No identification of feather with an individual bird was possible. The white under-wing covert must have been molted by an old sub-adult or adult condor. The white feather and the cabinetmaker's plane used for shaving fine samples from the rachis of feathers are shown in Figure 4.

Figure 4. California Condor under-wing covert and cabinetmaker's plane used in preparation of feather samples for lead analysis.

Feathers were cleaned with a diluted solution of commercial non-ionic detergent (Ivory liquid) and rinsed under running deionized water for 2-3 minutes to remove feces and dirt that contaminated the surface of the molted feathers before recovery from the flight pens. Each feather was repeatedly saturated with running water, and then shaken vigorously to displace all the water from the feather. The effectiveness of the rinsing could be evaluated, because the surfactant quality of the detergent makes water "flow" along the feather, while detergent-free feathers are very water repellant. Deionized water beads-up on the surface of a completely rinsed, clean feather. When shaken, clean feathers dry almost immediately. Feathers were therefore rinsed in deionized water until the feathers were dry. A final series of rinses in deionized, glass-distilled water was carried out to reduce possible metal contamination of the feathers to a minimum.

Feathers were sampled by shaving a thin sliver of material from the entire length of the rachis with a miniature cabinetmaker's block plane. The total sample weight varied between feathers, due to differences in feather size, and differences in depth of cut with the plane. Most samples weighed between 0.23 and 0.40g, and represented an integrated sample from each part of the 3-4 month feather growth period. The white under-wing covert was too small to sample by this method, but could be sampled in the future by ICP-MS.

Table 1. Lead Content of Molted Condor Feathers
Hopper Mountain Facility, Dec. 2002

Feather Number	sample wt. (g.)	Lead Content (ug/g)	Minimum Detection Lilmit
1	0.26	ND	0.24
2	0.34	0.18	0.18
3	0.25	0.24	0.24
4	0.56	1.02	0.12
5	0.44	0.29	0.14
6	0.25	ND	0.24
7	0.3	ND	0.20
8	0.28	ND	0.24
9	0.34	1.81	0.18
10	0.3	0.71	0.20
11	0.26	ND	0.24
12	0.23	0.25	0.24
13	0.3	ND	0.20
14	0.39	0.22	0.15
15	0.25	ND	0.24
16	0.23	ND	0.24
17	0.25	ND	0.24
18	0.46	0.63	0.12
19	0.41	0.18	0.15
20	0.29	0.52	0.20
21	0.29	0.30	0.20
22	0.25	0.25	0.24
23	0.23	0.45	0.24
24	0.27	ND	0.24
25	0.28	ND	0.20
26	0.26	ND	0.24

For this study, ICP analysis performed by CAHFS was employed to evaluate molted condor feathers. Although the sensitivity is not as great as GFAAS or ICP MS, the low cost of analysis, and sufficient sensitivity to detect lead in samples as small as 0.25 g of feather made it suitable for the screening studies to determine lead exposure of condors in the wild.

Of the 26 feathers provided by the Hopper group, 14 had measurable lead content, and 12 feathers had undetectable levels of lead (minimum detection levels were 0.12-0.24 ppm., depending upon the size of the sample supplied to CAHFS (Table 1).

Two of the feathers contained over 1 ppm lead in the averaged sample (1.02, and 1.81 ppm). Three of the feathers contained between 0.5 and 1.0 ppm (0.52, 0.63, and 0.71 ppm). The remaining 9 feathers with detectable lead contained between 0.18 and 0.45 ppm Pb.

I expected that most of the molted feathers would be feathers that had been gown while the condors were in captivity. Because the molt cycle for primaries and secondaries is on a gradual two-year cycle, the first primary molt would occur when the birds are two years old. The feathers molted at the first molt would not be expected to contain measurable amounts of lead, if the food supplied to the birds at the breeding facilities at LA or San Diego Zoo were lead free.

Most condors have been released at the age of one or two years, and most of their first molt cycle would be expected to occur in the wild. The first molt cycle is expected to begin at about age two, and take most of the two years between age two and four. All of the feathers dropped and replaced in the first molt cycle would have been grown before fledging in the breeding facilities at San Diego or Los Angeles zoos. The second molt cycle is expected to begin about age four, and continue almost to age six. Thus, for a molted feather to have been grown in the wild, while the condors were feeding on wild or supplied carcasses, the feather would have to be from a bird at least 4 years old, which had been in the wild for the previous two years. The minimum time possible for a bird to be in the wild and provide a wild grown feather would be a bird released before age two, and in the field for about two years. All feathers molted after a bird had been in the wild for two years would be expected to have been grown in the wild.

Elevated levels of lead in feathers presented in Table 1 probably did not represent an even deposition of Pb throughout feather growth, but probably represent one or more lead exposure events during the 3-4 month period of feather growth. A single exposure and depuration episode might represent only 10-25 days, making the exposure only a small fraction of the total feather growth period, perhaps 10-20% of the 105-120 day feather growth period. Two of the feathers contained 1.02-1.81 ppm lead, which could represent a substantial lead exposure for a short duration, perhaps levels of 10-20 ppm deposited into daily feather segments.

The sequence of lead deposition along the length of a feather needs to be quantified at high resolution to be able to test this hypothesis. All of the feathers analyzed in this study have been delivered to Don Smith and Molly Church at UCSC for further analysis. The section on future work gives an example of the results I expect could be obtained from both molted feathers and from feathers taken at necropsy for lead analysis.

Analysis of Condor Carcass Blood Feathers

Objective 4 of this study was to examine blood feathers of dead condors that have been stored frozen. Bruce Palmer and Bob Risebrough collected feathers during October 2002 expressly for lead determinations. I obtained a single feather from Condor 191 in March, 2003, just before the closure of this study. Condor 191 was a particularly good bird to

examine, because she was discovered alive, but in very poor condition in Arizona on June 15, 2000, and she died June 16, 2000. Condor 191 weighed only 4.9 kg (about 60% of normal weight), and had 17 ppm Pb in her liver at necropsy.

Especially important for this study was the fact that Condor 191 had been previously captured in mid April 2002, and was blood-sampled for lead on April 20, 2000, at which time her blood lead was 14 ug/dl. This was 81 days prior to her death, and it is probable that the April lead exposure event could have been "recorded" in the growing feather present at death of Condor 191 in June 2000.

Unfortunately, the protocol I specified for the collection of all of the feathers from carcasses was not followed, and I was not allowed to participate in the feather collection to insure correct collection. Each of the growing feathers was cut off at the wing margin, rather than carefully dissecting the follicle out of the wing tissue to collect the growing follicle. It appeared from my inspection of the primary from Condor 191 that approximately 6-8 cm of feather follicle was left in the wing, which would represent approximately two weeks of feather growth immediately prior to death of the bird.

The two weeks duration of feather growth prior to the deaths of each of the archived condors was critical for the determination of lead exposure to the birds. It is truly disappointing that these most important feathers were collected incorrectly. For the lead exposure study to be conducted in the future, the frozen carcasses will again have to be thawed, and the cut feather follicles will have to be carefully dissected out.

D. Extrapolation of lead analysis data for future work.

The analysis of methods to detect lead, and the preliminary analysis of condor feathers indicates that high resolution detection of lead during feather growth is currently possible, and that much information on lead exposure of condors could be gained from further analysis of feathers. Valuable information could be obtained from analysis of both molted feathers, and feathers from carcasses of condors currently in possession of the USFWS condor Program.

Figure 5 gives an example of possible results for such an analysis of a single growing or molted feather. The hypothetical feather in the figure represents a condor that was exposed twice to lead about 50 days apart. The feather depicted has been cut lengthwise to archive half of the feather, and one half has been divided into 50 sections of about 1 cm length, representing about 2 days of feather growth. Each small segment would contain about 50 mg of material. If these 50 mg samples were analyzed by GFAAS, a minimum level of about 0.15 ppm lead could be detected. If analyzed by ICP-MS, a detection limit of 0.01 ppm could be expected, as well as the determination of the lead isotope ratios. The lead isotope ratios could potentially differentiate between atmospheric lead or lead from an ingested bullet fragment.

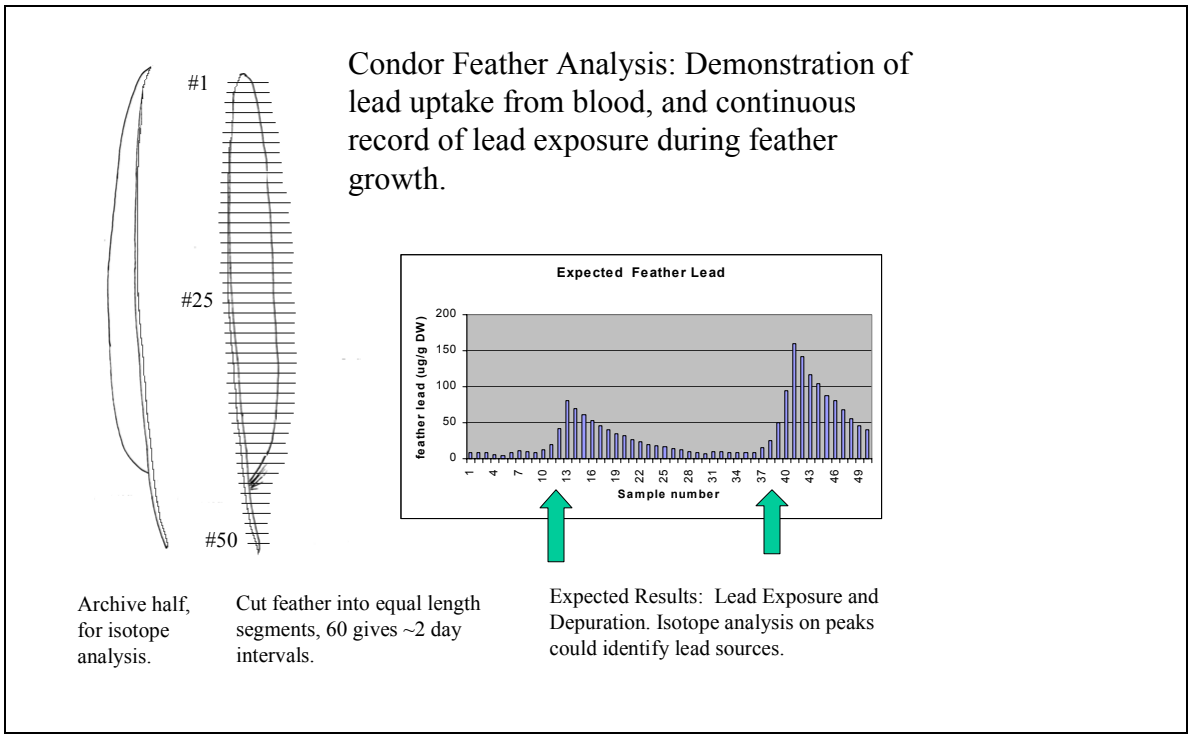


Figure 5. Hypothetical feather analysis. Flight feather is split longitudinally and half sectioned for analysis of fractions. Sample fraction numbers are given, and expected results of a feather from a bird exposed twice to lead, at the arrows, are given at right.

Analysis of molted feathers from condors that have been in the field for more than two to four years would give the frequency and relative severity of lead exposure incidents.

Analysis of feathers that were growing at the time of death of condors in the field should provide information on the time-course of lead exposure for those birds known to have been exposed to lead, and would provide lead exposure data for those birds scavenged after death, for which there is no toxicological information on cause of death. Liver lead residue data exists for 18 of 35 birds examined at necropsy, and does not exist for 17 birds. This study would confirm the lead exposure for those birds known to have died from lead, and would provide the best correlation between liver and feather lead levels. Results of analysis of blood feathers from archived condors would provide data on lead exposure during the weeks prior to death of condors recovered from the wild. Data from the 17 birds without toxicological data would fill that important data gap, and give a more complete analysis of the causes of condor mortality.

I recommend that these studies be continued and that the feather follicles that were cut off in the feather collection of October 2002 be retrieved and analyzed to be able to document the potential lead exposure of all recovered condor carcasses.

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