

Ammunition is the Principal Source of Lead Accumulated by California Condors Re-Introduced to the Wild

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The endangered California Condor (*Gymnogyps californianus*) was reduced to a total population of 22 birds by the end of 1982. Their captive-bred descendants are now being released back into the wild in California, Arizona, and Baja California, where monitoring indicates they may accumulate lead to toxic levels. Fragments of ammunition in the carcasses of game animals such as deer, elk, and feral pigs not retrieved by hunters or in gut piles left in the field have been considered a plausible source of the lead, though little direct evidence is available to support this hypothesis. Here, we measured lead concentrations and isotope ratios in blood from 18 condors living in the wild in central California, in 8 pre-release birds, and in diet and ammunition samples to determine the importance of ammunition as a source of exposure. Blood lead levels in pre-release condors were low (average 27.7 ng/mL, SD 4.9 ng/mL) and isotopically similar to dietary and background environmental lead in California. In contrast, blood lead levels in free-flying condors were substantially higher (average 246 ng/mL, SD 229 ng/mL) with lead isotopic compositions that approached or matched those of the lead ammunition. A two-endmember mixing model defined by the background ²⁰⁷Pb/²⁰⁶Pb ratio of representative condor diet samples

(0.8346) and the upper ²⁰⁷Pb/²⁰⁶Pb ratio of the ammunition samples (0.8184) was able to account for the blood lead isotopic compositions in 20 out of the 26 live condors sampled in this study (i.e., 77%). Finally, lead in tissues and in a serially sampled growing feather recovered post-mortem from a lead-poisoned condor in Arizona evidence acute exposure from an isotopically distinct lead source. Together, these data indicate that incidental ingestion of ammunition in carcasses of animals killed by hunters is the principal source of elevated lead exposure that threatens the recovery in the wild of this endangered species.

Introduction

The range of the California Condor (*Gymnogyps californianus*) extended across much of the current United States during the Pleistocene epoch (1–3). Obligate scavengers, California Condors typically feed on the carcasses of large mammals. After the extinction of the Pleistocene megafauna, their range was restricted to the west coast, where marine mammals were a significant source of food (4–6) until hunting decimated the whale and pinniped populations. The creation of large cattle and sheep ranches provided new food resources (5, 7), though shooting and the poisoning of carcasses for predator control greatly reduced condor numbers, so that by the end of the nineteenth century the condor was already considered a “doomed bird” (8). In the early 1980s the species approached extinction; by the end of 1982 only 22 birds survived, three of which were in captivity (9, 10). By early 1986, 11 of the birds in the wild had died (9, 10). Lead poisoning was attributed as the cause of death of three of the four birds that were recovered (11, 12), prompting the controversial decision to bring all birds into captivity (10). The success of the captive breeding programs at the Los Angeles Zoo, the Wild Animal Park of the San Diego Zoological Society, and the World Center for Birds of Prey has led to a reintroduction program to the wild. As of June 1, 2006 there were 42, 23, 58, and 12 condors in the wild in Central California, Southern California, Arizona, and Baja California, respectively (13).

An analysis of counts and other observations of California Condors between 1940 and 1987 indicated that the population decline over this period was due to excessive adult mortality of this long-lived species due to shooting, lead poisoning, and other factors (9), rather than reproductive failures such as those caused by the DDT compound DDE (14, 15). Shooting had been a particularly significant factor in the earlier years, while not until the 1980s was lead poisoning recognized as a potentially significant cause of mortalities (9, 11). Fragments of ammunition in the carcasses of hunted animals such as mule deer (*Odocoileus hemionus*), elk (*Cervus elaphus*), wild pigs (*Sus scrofa*), and other mammals not retrieved by hunters or in gut piles left in the field have been considered to be the plausible source of the lead exposure (9, 12). Hunting remains a popular activity in California; in 2001 over 100,000 large game animals were killed by hunters in the eight California counties where condors now range (16). A bullet fragment was found in the ventriculus of one of the three condors that died of lead poisoning in the 1980s, a metallic object was detected in the ventriculus of another, but the third had no detectable metal fragments (11, 12).

Blood lead levels of California Condors have been monitored since the beginning of the reintroduction program in 1992 (17, 18). Over 1997–2000 four birds with blood lead

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levels of 1850 ng/mL or higher were treated with chelation therapy; two survived and later bred in the wild, but the other two later disappeared with no determined cause of death. Within the same time interval six blood lead values between 500 and 1000 ng/mL were recorded in condors in southern California. The vast majority of birds released to the wild acquired somewhat lower blood lead levels after leaving the release areas (17). Still, many blood lead values exceeded by several-fold or more levels known to be toxic to humans (19–21) and to a range of other species (22–26).

We hypothesized that the most likely source of elevated lead for condors released in California was inadvertent ingestion of spent ammunition fragments embedded within animal carcasses shot with lead ammunition. To test this hypothesis, we measured lead concentrations and stable lead isotopic compositions in whole blood samples from reintroduced ($n = 18$) and pre-release ($n = 8$) California Condors, post-mortem tissues from a condor who died from lead poisoning in Arizona, and in ammunition and representative condor diet samples. Since the relative abundances of the naturally occurring isotopes of lead (^{204}Pb , ^{206}Pb , ^{207}Pb , and ^{208}Pb) typically vary among different industrial materials, the lead isotopic fingerprint of lead-exposed organisms will reflect the isotopic fingerprint of the lead exposure source, thereby providing a powerful tool to determine sources of lead exposure to organisms (27–29). At the time of the study, the sample size of live condors sampled for this study represented 82% of the wild condor population in central California, 43% of the wild population in all of California, and ~20% of the total wild population, including condors in Arizona and Baja California (13).

Materials and Methods

Sample Collection and Processing. *Live Condors.* Whole blood samples were collected opportunistically from free-flying condors in central California ($n = 18$) by Ventura Wildlife Society crew members between October 2002 and June 2004, and from flight pen-stationed condors awaiting release at the Hopper Mountain Reserve in southern California ($n = 8$) by USFWS personnel in March 2003 and June 2004. Free-flying condors were trapped using walk-in traps baited with still-born calves or live condors. Within the traps condors were netted and gently restrained while blood was collected from the tibial artery directly into low-lead Vacutainers containing sodium heparin, using a 21-gauge catheter and established procedures to minimize sample lead contamination (30). Samples were stored on ice for transport to the laboratory where they remained frozen at $-20\text{ }^{\circ}\text{C}$ until analyzed.

Condor 165 Post-Mortem Tissues. To demonstrate the utility of using growing feathers to reveal temporal changes in the magnitude and source(s) of lead exposure to condors, we analyzed a growing retriex feather, kidney, liver, and bone samples recovered post-mortem from Condor no. 165. Condor 165 was hatched at The Peregrine Fund's Birds of Prey Center in Boise, Idaho on April 20, 1997, released at Vermillion Cliffs in Arizona on November 20, 1997, and was recovered dead on June 12, 2000. The condor necropsy performed at the pathology laboratory of the Zoological Society of San Diego found 16–17 lead shotgun pellets in the ventriculus (gizzard), which were later misplaced and unavailable for isotopic analyses. Death was attributed to lead poisoning based on elevated kidney and liver lead concentrations of 96 and 34 $\mu\text{g/g}$ wet weight, respectively (31). Aliquots of liver and kidney frozen at $-20\text{ }^{\circ}\text{C}$ were provided to this study. A retriex feather measuring 23.8 cm long that was growing at the time of death and a sample of the tibiatarsus were collected for use in this study from the preserved study skin at the Western Foundation of Vertebrate Zoology (Camarillo, CA).

Condor Diet Samples. Liver, kidney, and/or rib bone samples from representative condor diet samples were collected, including samples from two calves (*Bos taurus*) from dairy farms in Monterey and Kern Counties, California, two road-killed mule deer (*Odocoileus hemionus*) from Monterey and Ventura Counties, California, and one California sea lion (*Zalophus californianus*) that washed up in Santa Cruz County, California. Samples were dissected from the intact carcass and stored frozen at $-20\text{ }^{\circ}\text{C}$ until analyzed.

All biological samples (blood, soft tissue, bone, feather) were prepared, processed, and analyzed in duplicate using established trace metal clean techniques under HEPA filtered-air laboratory conditions (28, 30). Laboratory water was ultrapure grade (Milli-Q system, $18\text{ M}\Omega\text{-cm}^2$), and acids were sub-boiling quartz double-distilled grade (Fisher Scientific). All laboratory ware was acid cleaned following procedures detailed elsewhere (30). Blood samples were thawed and the sample was thoroughly mixed by repeated gentle inversion and brief vortexing. A 0.25–1.0 mL aliquot of blood was processed for analyses as previously described (28). Liver and/or kidney samples from condor 165 and from representative condor diet samples were partially thawed, and the starting sample (typically measuring $\sim 1\text{--}2\text{ cm}^3$ and 0.75–1 g) exterior was dissected away to yield a final sub-sample from within the starting sample. For condor 165 bone samples, a 3–4 cm (0.17–0.25 g) segment of tibiatarsus was cleaned of all surficial tissue; 1–2 cm subsections of tibiatarsus were then bisected longitudinally, the outer and inner surfaces were cleaned of remaining periosteum, marrow, and endosteum, and the samples were rinsed sequentially with 0.15 M nitric acid and ultrapure water. For diet bone samples (calf, deer, sea lion), a 1 cm section of rib was cleaned of surficial tissue and processed as above. All samples were dried at $65\text{ }^{\circ}\text{C}$ to a constant weight and digested overnight with 2 mL of sub-boiling concentrated (15 M) nitric acid in closed Teflon vials, evaporated to dryness, and diluted with 1 M nitric acid for analyses. Bone samples were additionally processed using anion exchange column chemistry with BioRad AG1-X8 anion resin to separate sample lead from the bone mineral matrix (28).

The retriex feather from condor 165 was processed in the clean-lab to remove surficial contaminant lead. The feather was washed using a dilute phosphate-based detergent and rinsed with ultrapure water, sonicated for 5 min in ultrapure water, rinsed with HPLC grade ethanol, rinsed with ultrapure water, rinsed with 0.15 M nitric acid, and rinsed a final time with ultrapure water. The feather was then dried overnight at $65\text{ }^{\circ}\text{C}$ to a constant weight under HEPA filtered-air conditions. Subsequently, the feather was sub-sampled for analyses by cutting the rachis (shaft) cross-sectionally into sequential 2 cm sections using a stainless steel scalpel, with each section containing intact rachis and attached feather vane. Subsequently, each 2 cm vane section was cut from either side of the rachis for digestion, and two sequential 2 cm rachis sections were pooled for digestion. This sub-sampling scheme produced six sections of rachis (4 cm each) and ten sections of vane spanning the feather (the most distal rachis sample yielded a very small piece, 8 mg, due to the shape of the feather at its tip, which was combined with the adjacent proximal vane sample) (see Figure 2). The range in weights for these samples was 17–232 mg.

Ammunition. Remington and Winchester ammunition were purchased from different Big 5 and Kmart stores in three cities in San Luis Obispo and Ventura Counties. These locations serve hunters within the foraging range of California Condors. In total, 13 boxes of ammunition were purchased: three boxes of 12 gauge shotgun ammunition (two from San Luis Obispo County and one from Ventura County), three boxes of 30-06 rifle bullets (two from San Luis Obispo County and one from Ventura County), four boxes of 30-30 rifle bullets

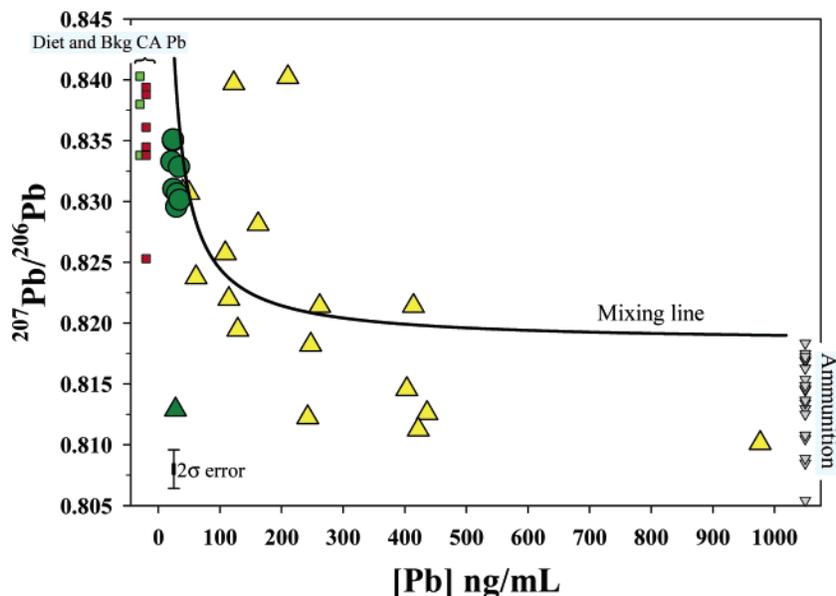


FIGURE 1. Lead concentrations versus $^{207}\text{Pb}/^{206}\text{Pb}$ ratios in blood of free-flying (triangles) or pre-release (circles) California Condors. Blood lead isotopic compositions from condors with low lead levels (green ●, defined here as ≤ 37.5 ng/mL, $n = 9$, see text) are similar to the lead isotopic composition of representative diet items (red ■) condors are provided (stillborn dairy calves) or are known to feed on in the wild (mule deer, *Odocoileus hemionus*, California sea lions, *Zalophus californianus*) and to the isotopic composition of background environmental lead in California (green ■, 28, 38–40). The blood lead isotopic composition of most condors with elevated blood lead levels (>37.5 ng/mL, yellow ▲, $n = 17$) shifts away from the diet isotopic composition and toward lead ammunition used in the condor's central California range (gray ▼). The one exception (green ▲) likely reflects a condor previously exposed to an ammunition lead source whose body lead burden has since returned to background levels but retained the isotopic signature of the elevated exposure. The mixing line, derived using the equation $Y = (37.5 \times 0.8346 + (X - 37.5) \times 0.8184)/X$, shows the calculated lead isotopic composition of condor blood containing background lead from their representative diet (average $^{207}\text{Pb}/^{206}\text{Pb} = 0.8346$) mixed with increasing amounts of lead from ammunition ($^{207}\text{Pb}/^{206}\text{Pb} = 0.8184$ was used in this calculation). Blood isotopic compositions that fall on or left of/below the mixing line are consistent with the mixing of lead from diet and ammunition sources. Twenty out of the 26 condors sampled in this study (i.e., 77%) contained blood lead isotopic compositions that were consistent with this two end-member mixing model. The error bar on the lower left is the two sigma (SD) measurement error for lead isotopic compositions and concentrations by ICP–MS.

(three from San Luis Obispo County and one from Ventura County), and three boxes of 0.270 rifle bullets (two from San Luis Obispo County and one from Ventura County). In addition, nine rifle bullets (30-06, 30-30, 0.270 caliber) were obtained from recreational hunters in Santa Cruz, Santa Clara, and Plumas Counties. Six bullets or shells were selected from each box, and processed as two samples per ammunition box. Each sample analyzed was composed of pooled leaches from three bullets/shells. The tips of the bullets were pinched off and collected, and five pellets were taken from each shotgun shell. These bullet tips/pellets were rinsed sequentially with ultrapure water, methanol, ultrapure water, and 1 M nitric acid to clean the surface. The individual fragments were then immersed in a 1 M nitric bath for 1 min, rinsed with ultrapure water, and then put into a polyethylene vial containing 2 mL of 1 M nitric acid and shaken by hand for 2 min. The fragments were removed, and the 2 mL leachates from each of the three bullets/shells were combined. The nine donated lead ammunition bullet samples were processed similarly, except that they were processed individually rather than as pooled leachates. Leachate samples were screened using flame atomic absorption spectrometry to estimate sample lead concentrations, and then diluted to 25–50 ng/mL and analyzed by ICP–MS for lead concentration and isotope ratios, as described below.

Sample Analysis. Lead concentrations and isotopic ratios were determined in all samples using a Finnigan MAT Element magnetic sector–inductively coupled plasma mass spectrometer (ICP–MS), measuring masses of ^{206}Pb , ^{207}Pb , ^{208}Pb , and ^{209}Bi . Bismuth-209 (^{209}Bi) was added as an internal standard to correct for short-term sample-to-sample fluctuations in instrument sensitivity for the lead concentration measurements. External standardization for lead isotopes

was made using the National Institute of Standards and Technology (NIST) standard reference material 981 (SRM 981) lead isotopic standard, and cross calibrated using a digested blood sample that had been independently characterized for isotopic composition using a VG Sector thermal ionization mass spectrometer (32).

Samples were diluted to 10 ng/mL when analyzed by ICP–MS in counting mode and to 25–50 ng/mL when analyzed in analogue mode. The SRM 981, which had previously been cross-calibrated for lead concentration with a certified lead standard (Spex Industries Incorporated, Edison, NJ), was used to calculate the lead concentrations and lead isotopic compositions of the samples (29, 32). Accuracy of the lead concentration measurements in biological samples was 98.6%, based on measurements of NIST SRM 955b (lead in bovine blood) over the course of study, while the precision of lead concentration measurements was 1.3% ($2 \times$ relative standard deviations, 2 RSD). Precision of lead isotopic measurements was 0.20% (2 RSD) for $^{207}\text{Pb}/^{206}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ ratios, based on triplicate measures of condor tissue samples (blood, liver, or kidney) made on each of the nine analysis days.

Intercalibration and validation of the lead isotopic ratio measurements of this ICP–MS method has been demonstrated previously via comparison with isotopic measurements by thermal ionization mass spectrometry (32). To again demonstrate the accuracy of the ICP–MS isotopic measurements made here, six condor blood samples were processed using BioRad AG1-X8 anion resin column separation of sample lead (28), and analyzed using a ThermoFinnigan Neptune double-focusing multicollector (MC) magnetic sector ICP–MS (MC–ICP–MS). Results of this intercalibration (data not shown) showed that the $^{207}\text{Pb}/^{206}\text{Pb}$ ratios

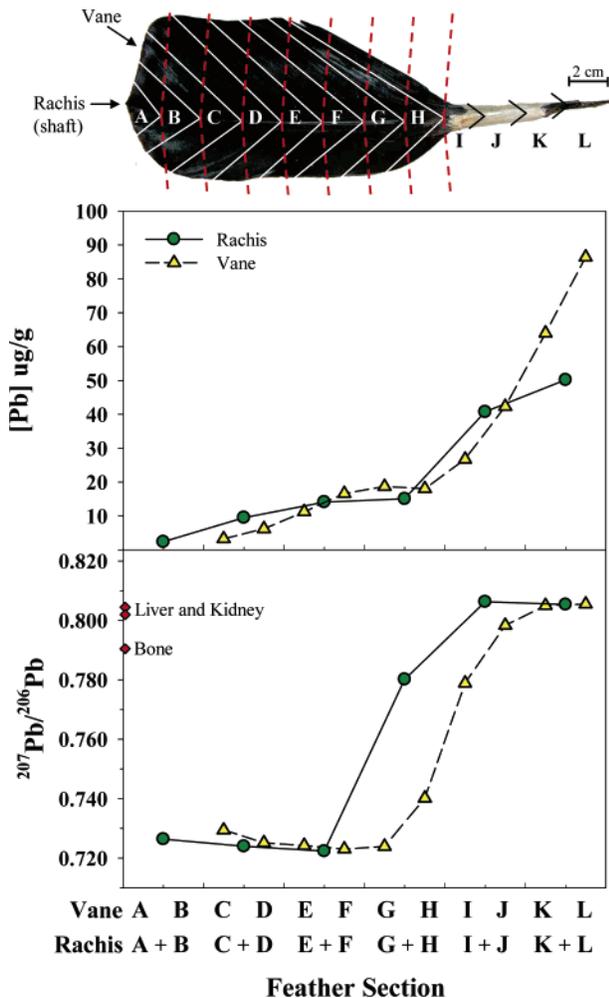


FIGURE 2. Lead concentrations and lead isotopic compositions of a retrix feather rachis (shaft) and vane from condor 165 whose death in Arizona was attributed to lead poisoning. Lead concentrations and isotopic compositions of the feather rachis and vane segments (designated A–L) changed along its 24 cm length consistent with abrupt exposure to an elevated lead source over the time of feather growth. Feather vane samples were composed of 2 cm sections (sections C, D, E, etc.) cut from associated rachis, while rachis samples were composed of two sequential 2 cm sections (i.e., A + B, C + D, etc.). Vane samples (approximate) are delineated by the white chevron lines on the feather, while the estimated feather growth isochrones are delineated by red-dashed lines, according to Prum and Williamson (49). Lead isotopic compositions of liver (27.4 $\mu\text{g/g}$ wet wt), kidney (74.1 $\mu\text{g/g}$ wet wt), and bone (6.5 $\mu\text{g/g}$ dry wt) tissue from this condor are plotted on the left y-axis. This growing feather was sampled from the follicle. [Note: Rachis and associated vane extend to the younger proximal tip (right side) of the feather, though they are covered by a sheath over sections I to L in this picture.]

measured on the Element ICP–MS and Neptune MC–ICP–MS were in close agreement, with an average percent difference of 0.04%—well within the 2 RSD error of the Element ICP–MS measurements determined over the course of the study (0.2%).

It was considered that lead exposures to condors in California might also coincide with elevated exposures to other toxic metals originating from unknown environmental source(s). To evaluate this, a subset of 11 condor blood samples were analyzed for arsenic (As), silver (Ag), cadmium (Cd), chromium (Cr), copper (Cu), selenium (Se), zinc (Zn), and lead (Pb) by ICP–MS in low (Ag, Cd, Pb), medium (Cr, Cu, Zn), or high (As, Se) resolution, measuring masses ^{109}Ag ,

TABLE 1. Lead Concentrations and Isotopic Compositions of Blood Samples Collected from Free-Flying Condors in Central California between October 2002 and June 2004 (Free-Flying, $n = 18$), and from Condors Awaiting Release from Flight Pens at the Hopper Mountain Reserve in Southern California in March 2003 and June 2004 (Pre-Release, $n = 8$)

Condor ID ^a	status at collection	[Pb] ng/mL ^b	$^{207}\text{Pb}/^{206}\text{Pb}$	$^{208}\text{Pb}/^{206}\text{Pb}$
294	pre-release	24.5	0.8310	2.036
298	pre-release	33.5	0.8329	2.040
301	pre-release	34.5	0.8301	2.038
303	pre-release	29.2	0.8296	2.034
306	pre-release	24.0	0.8350	2.047
311	pre-release	24.0	0.8351	2.044
317	pre-release	21.3	0.8333	2.047
318	pre-release	30.3	0.8307	2.037
161	free-flying	242	0.8122	2.002
164	free-flying	129	0.8195	2.020
167	free-flying	61.2	0.8238	2.022
168	free-flying	109	0.8257	2.027
170	free-flying	436	0.8126	2.005
171	free-flying	114	0.8220	2.025
190	free-flying	122	0.8397	2.049
192	free-flying	210	0.8402	2.057
199	free-flying	977	0.8101	2.000
204	free-flying	262	0.8214	2.024
209	free-flying	422	0.8112	2.003
212	free-flying	403	0.8146	2.008
219	free-flying	414	0.8214	2.018
222	free-flying	162	0.8281	2.038
231	free-flying	247	0.8182	2.015
236	free-flying	28.0	0.8129	1.996
242	free-flying	39.6	0.8308	2.038
251	free-flying	49.5	0.8307	2.033

^a Condor ID number assigned by California Condor Studbook Keeper, San Diego Wild Animal Park. ^b Reported concentration and isotope ratio values are averages of duplicate aliquots from a single blood sample that differed by <2% in concentration measurements, <0.001% for $^{207}\text{Pb}/^{206}\text{Pb}$ measurements, and <0.03% for $^{208}\text{Pb}/^{206}\text{Pb}$ measurements. Reproducibility of the isotope ratio measurements is $\pm 0.20\%$ 2RSD, which is equivalent to 0.002 error (2SD) for a $^{207}\text{Pb}/^{206}\text{Pb}$ ratio of 0.830.

^{75}As , ^{110}Cd , ^{52}Cr , ^{63}Cu , ^{82}Se , ^{66}Zn , and ^{208}Pb , with added rhodium (^{103}Rh) and bismuth (^{209}Bi) as internal standards. External standardization was via certified standards (Spex Industries, Inc.). The analytical detection limits (in ng/mL) for the above elements were as follows: Ag, 0.008; Cd, 0.533; Cr, 0.128; Cu, 0.040; Zn, 21.3; As, 0.074; and Se, 5.23. Standard reference materials (NIST SRM 955b, and 1577b, bovine liver) and blood spike-recoveries were used to confirm analytical accuracy, which was $101\% \pm 3\%$ RSD for lead concentrations measurements, and $95\text{--}105\% \pm 7\text{--}12\%$ RSD for concentration measurements of the other elements listed above.

Results and Discussion

Condor blood lead levels ranged from 21.3 to 977 ng/mL (median 112 ng/mL, average 179 ng/mL, SD 215 ng/mL), though levels in the 18 free-flying condors were substantially higher (median 186 ng/mL, average 246 ng/mL, SD 229 ng/mL) than in the pre-release flight pen-stationed birds (median 26.9 ng/mL, average 27.7 ng/mL, SD 4.9 ng/mL). The blood $^{207}\text{Pb}/^{206}\text{Pb}$ isotope ratios also varied greatly among the birds, ranging from 0.8101 to 0.8402 (Table 1, Figure 1). We determined background non-elevated blood lead levels in these California Condors to be less than or equal to 37.5 ng/mL, based on the average pre-release condor blood lead level + 2 SD (i.e., $27.7 + 9.8 \text{ ng/mL} = 37.5 \text{ ng/mL}$). The eight pre-release condors with background blood lead levels had $^{207}\text{Pb}/^{206}\text{Pb}$ isotope ratios significantly different from those of condors with blood lead levels above 37.5 ng/mL ($p = 0.007$, Kruskal–Wallis). Furthermore, blood lead levels and

lead isotope ratios were strongly inversely associated with each other. The nonparametric Spearman ρ measure of association, which is independent of the functional relationship between the two variables, is highly significant ($\rho = -0.6745$, $p < 0.001$). This association between lead concentration and lead isotope ratios in blood is consistent with exposure of the condors after release to an elevated lead source with an isotope ratio different from their pre-release background environmental lead, leading to elevated blood lead levels with lower $^{207}\text{Pb}/^{206}\text{Pb}$ isotope ratios compared to non-exposed condors (Table 1, Figure 1).

The background blood lead level of ≤ 37.5 ng/mL for condors in California is consistent with recent studies showing that non-lead-exposed raptors and seabirds possess low blood lead levels comparable to current background levels in humans (e.g., < 50 ng/mL or $5 \mu\text{g}/\text{dL}$) (33–35). This background condor blood lead level is substantially lower than the background blood lead criteria of < 200 ng/mL ($20 \mu\text{g}/\text{dL}$) proposed for waterfowl (36) and Falconiformes (37). However, those latter criteria may not reflect current background lead levels in avian wildlife, since they were based on studies conducted several decades or more ago when environmental lead exposures were higher than they are today, and before there was widespread recognition of the precautions necessary to avoid sample lead contamination during collection, processing, and analyses (28, 30).

To determine whether inadvertent ingestion of spent ammunition fragments embedded within mammal carcasses shot with lead ammunition was the principal source of elevated exposures to released condors, we measured lead isotope ratios in lead ammunition used to hunt game and other animals in the condors' California habitat. We also measured lead concentrations and isotope ratios in stillborn dairy calves that are fed to condors awaiting release and after release into the wild, and in tissues of large mammals (mule deer, California sea lion) that condors are known to feed on in the wild. The representative diet samples contained very low lead concentrations (liver lead levels ranged from 2 to 68 ng/g dry wt, or 0.3 to 15 ng/g wet wt; bone lead ranged from 38 to 190 ng/g dry wt), and possessed $^{207}\text{Pb}/^{206}\text{Pb}$ isotope ratios (average 0.8346 ± 0.0046 SD, $n = 7$) similar to the $^{207}\text{Pb}/^{206}\text{Pb}$ isotope ratios of background environmental lead in California (0.8338–0.8453) (Table 2) (28, 38–40). In contrast, ammunition samples ($n = 18$ different brands and calibers) had much lower $^{207}\text{Pb}/^{206}\text{Pb}$ ratios ranging from 0.8054 to 0.8184 (median 0.8145, average 0.8136 ± 0.0035 SD) (Table 3) that were statistically different from the diet samples ($p < 0.001$, Kruskal–Wallis).

Condors with background blood lead levels (i.e., ≤ 37.5 ng/mL) possessed blood $^{207}\text{Pb}/^{206}\text{Pb}$ ratios that were, with one exception, similar to the diet $^{207}\text{Pb}/^{206}\text{Pb}$ ratios, whereas condors with higher blood lead concentrations generally had isotope ratios that approached those of the ammunition (Figure 1). This trend was compared with a two end-member mixing model hypothesized to account for the blood lead isotope ratios measured in these condors. The model is described by the mixing line plotted in Figure 1, using for the "background" end-member a blood level of 37.5 ng/mL and blood $^{207}\text{Pb}/^{206}\text{Pb}$ ratio of 0.8346 (i.e., the diet average), and for the elevated lead exposure end-member the upper $^{207}\text{Pb}/^{206}\text{Pb}$ ratio of the ammunition samples (0.8184). Blood $^{207}\text{Pb}/^{206}\text{Pb}$ ratios that fall on or left of/below the mixing line are consistent with this model, since after cessation of elevated lead exposure blood lead concentrations would have decreased with time while the lead isotope ratio would have changed very little until the new body lead stores were markedly depleted and replaced with background environmental lead. Twenty out of the 26 condors sampled in this study (i.e., 77%) possessed blood lead isotope ratios that were consistent with this two end-member mixing model,

TABLE 2. Lead Isotopic Compositions of Representative Condor Diet Samples Collected from Various Counties in California (Published Isotopic Values of Background Environmental Lead in California Are Shown for Comparison)

diet sample	collection location (County)	$^{207}\text{Pb}/^{206}\text{Pb}^a$
dairy calf	Monterey	0.8394
dairy calf	Monterey	0.8388
dairy calf	Kern	0.8253
dairy calf	Kern	0.8343
mule deer	Monterey	0.8338
mule deer	Ventura	0.8345
california sea lion	Monterey	0.8361
background environmental lead		
Sierra Nevada atmospheric dust (38)		0.8403
Sierra Nevada snow fed lake water (38)		0.8453
Northern California urban aerosols (39)		0.8403
Northern California environmental lead (28)		0.8380
Sacramento/San Joaquin river water (1998) (40)		0.8338

^a Isotopic values for each diet sample animal (dairy calves, mule deer, sea lion) were determined by analyzing both liver and bone samples from each animal. Tissue lead concentrations were all low; liver lead levels ranged from 2 to 68 ng/g dry weight or 0.3 to 15 ng/g wet weight, and bone tissues ranged from 38 to 190 ng/g dry weight.

TABLE 3. Lead Isotopic Composition of Lead Ammunition Samples Purchased from Retailers in California

ammunition type	manufacturer ^a	$^{207}\text{Pb}/^{206}\text{Pb}^b$
.270 rifle	Remington ^A	0.8169
.270 rifle	Winchester ^B	0.8134
.270 rifle	Remington ^C	0.8172
.280 rifle	Winchester ^D	0.8105
.380 rifle	Remington ^E	0.8085
30-06 rifle	Remington ^F	0.8054
30-06 rifle	Remington ^A	0.8175
30-06 rifle	Winchester ^B	0.8154
30-06 rifle	Remington ^C	0.8149
30-30 rifle	Remington ^F	0.8130
30-30 rifle	Winchester ^F	0.8145
30-30 rifle	Remington ^A	0.8163
30-30 rifle	Remington ^B	0.8089
30-30 rifle	Winchester ^B	0.8108
30-30 rifle	Remington ^C	0.8170
12 gauge shotgun	Remington ^A	0.8146
12 gauge shotgun	Winchester ^B	0.8137
12 gauge shotgun	Remington ^C	0.8125

^a Superscript letters indicate origin of sample: A, sporting goods store, Paso Robles, San Luis Obispo County; B, sporting goods store, Atascadero, San Luis Obispo County; C, sporting goods store, Ventura, Ventura County; D, donated by hunter in Plumas County; E, donated by hunter in Santa Clara County; F, donated by hunter in Santa Cruz County. ^b Values reported are averages of two composite leachate samples, each composed of three bullets/shotgun shells per ammunition box or averages of two leachate samples from each hunter-donated ammunition sample. Reproducibility of the isotope ratio measurements is $\pm 0.20\%$ 2RSD, which is equivalent to 0.002 (2SD) error for a $^{207}\text{Pb}/^{206}\text{Pb}$ ratio of 0.810.

supporting the hypothesis that the primary sources of lead to released California Condors are low background environmental lead in their diet and elevated levels of lead from ammunition, the latter most likely originating from the inadvertent ingestion of spent ammunition fragments embedded within mammal carcasses and offal piles. Hunted game and other mammals may contain hundreds of lead fragments (41, 42), the majority of which are likely to be small (< 1 mm), accounting for the condors with moderately elevated blood lead concentrations and an isotope ratio approaching that of ammunition (Figure 1).

TABLE 4. Concentrations of Metals (ng/mL) in Blood from Free-Flying California Condors (*n* = 11)^a

metal	median	mean	(SD)	range ^b
As	0.60	1.10	(1.80)	<DL–7.10
Ag	0.052	0.20	(0.42)	<DL–1.30
Cd	2.30	2.90	(1.70)	1.50–7.10
Cr	1.60	1.90	(1.00)	0.79–4.30
Cu	270	280	(52.0)	230–410
Se	900	930	(220)	680–1500
Pb	330	370	(240)	77–1050
Zn	5500	5300	(540)	4400–6000

^a No correlations were found between lead concentrations and concentrations of any of the other metals measured. ^b Except for lead, blood levels of these elements were within the normal physiological range (Cu, Zn), or within the range of values expected from background environmental exposures (Ag, As, Cd, Cr, Se) (44). Analytical detection limits (DL, ng/mL) were as follows: Ag (0.008), As (0.074), Cd (0.533), Cr (0.128), Cu (0.040), Se (5.23), Pb (0.060), and Zn (21.3).

The analysis of a subset of condor blood samples for elements other than lead showed that those elements were all within the normal physiological range (Cu, Zn), or within the range of values expected from background environmental exposures (Ag, As, Cd, Cr, Se) (Table 4). Also, there is no correlation between blood lead concentrations and any of these other measured metals, suggesting that the source of elevated lead exposure was specific for lead and did not contain elevated amounts of these other metals. This further implicates lead ammunition as the predominant source of elevated exposure, since lead ammunition contains >99% lead and <1% of other alloying metals such as arsenic, copper, silver, and cadmium (43). Exposure to lead from industrial sources or hazardous waste sites would likely be accompanied by co-exposures to other elements as well (44).

Incidental ingestion of lead ammunition fragments associated with mammal carcasses would likely produce episodic acute lead exposures with a range of intensities, depending on the number and size of ingested fragments (41, 42). As noted above (31), the death of condor 165 was attributed to lead poisoning on the basis of elevated lead concentrations of 96 and 34 $\mu\text{g/g}$ (wet weight) in the kidney and liver, respectively, and the presence of 16–17 shotgun pellets in the ventriculus. We measured kidney and liver lead concentrations of 74.1 and 27.3 $\mu\text{g/g}$ (wet wt, or 267 and 81.6 $\mu\text{g/g}$ dry wt), respectively, both well above expected background lead levels of <1 $\mu\text{g/g}$ (36, 37, 45, data from this study). Bone lead levels in this bird were 6.5 $\mu\text{g/g}$ (dry wt), which is also elevated compared to bone lead levels of ~ 0.40 $\mu\text{g/g}$ dry weight (± 0.20 SD, *n* = 5) measured in young released or zoo-housed condors with no known history of lead exposure (data not shown). Biological residence times of lead are very different in soft tissues and bone of vertebrates due to different blood perfusion rates in these tissues. In mammals, soft tissues such as kidney and liver have relatively high blood perfusion rates compared to bone, leading to relatively faster rates of lead uptake and release and, therefore, shorter lead residence times compared with the skeleton (46). The elevated tissue lead concentrations in condor 165 are consistent with a recent lead exposure event before its death, i.e., to ingested ammunition, producing very elevated soft tissue lead levels but only moderately elevated bone lead levels.

To test whether lead isotope ratios in tissues changed significantly due to known ingestion of lead shotgun pellets by condor 165, rachis and vane sections of a retrix feather that was growing at the time of death were serially sampled along the feather's 24 cm length. Since condor feathers can grow ~ 5 mm/day, they may provide a detailed record of the bird's lead exposure history over the period of feather growth, which may be several months (47). Lead concentrations were

lowest in the older (distal) part of the feather rachis and vane, and sharply increased to higher values in the younger (proximal) part of the feather (Figure 2). The proximal rachis and vane sections of the feather (I–L, Figure 2) have $^{207}\text{Pb}/^{206}\text{Pb}$ ratios that approach or match those of the kidney and liver samples collected post mortem, all of which are very different isotopically from the older, distal part of the feather (sections A–F, Figure 2). The exposure to an elevated and isotopically different lead source appears in rachis section G+H, as indicated by the sharp change in $^{207}\text{Pb}/^{206}\text{Pb}$ ratios. The change in lead concentration is not as immediate, probably reflecting the toxicokinetics of lead incorporation into maturing keratinocytes, which is a carrier-mediated regulated process. In contrast, the isotopic signature of the perfusing blood is readily recorded in the keratinocytes because biological processes controlling lead transport and uptake do not discriminate among lead isotopes. The abrupt change in vane $^{207}\text{Pb}/^{206}\text{Pb}$ ratios in sections H–J lag slightly behind the change in isotope ratios of comparable rachis sections (e.g., G+H, Figure 2). This is consistent with the presence of feather growth isochrones (points of equal time of origin), which extend out from the rachis to the vane as slightly inverted chevrons (Figure 2) (48, 49). Since vane samples were collected by cutting them from the rachis they integrate longer, earlier lead exposure periods compared to rachis samples from the same section.

The analysis of condor 165's feather demonstrates the feasibility of serial sampling of condor feathers to determine the timing, magnitude, and source(s) of lead exposure to free-flying condors. The $^{207}\text{Pb}/^{206}\text{Pb}$ ratios of ~ 0.806 in the proximal feather sections with the highest lead concentrations fall within the range of $^{207}\text{Pb}/^{206}\text{Pb}$ ratios of ammunition measured in this study (Figure 2, Table 3), and likely reflect the isotopic composition of the shotgun pellets ingested by condor 165. However, the apparent background $^{207}\text{Pb}/^{206}\text{Pb}$ ratios in the feather of condor 165 ($^{207}\text{Pb}/^{206}\text{Pb} \sim 0.735$, values measured in the oldest, distal tip of the feather), which was released and lived in Arizona, are very different from the background lead values for the live pre-release condors in California ($^{207}\text{Pb}/^{206}\text{Pb} \sim 0.834$) (Figures 1 and 2). Finally, other studies have reported feather lead levels as a biomarker of exposure and toxicity (e.g., 34, 50–53), though to our knowledge none have used serial sampling and stable isotopic analyses to determine the bird's lead exposure history.

In conclusion, our results indicate that incidental ingestion of ammunition embedded in carcasses that condors feed on is the principal source of elevated lead exposure that threatens the recovery of condors in the wild. The mixing model defined by representative natural diet samples and lead ammunition can explain most (77%) but not all of the blood lead concentration and isotopic composition values observed in the 26 condors studied here. Our ammunition samples, however, did not include all ammunitions sold in California, some of which might have the isotope composition of the two condor blood samples with isotope ratios slightly higher than background values (Figure 1). Detailed analyses of a growing condor feather substantiate the acute nature of lead exposure and accumulation in condors and demonstrate the utility of feathers as noninvasive biomarkers of lead exposure history in individual condors. In addition to mortalities from lead poisoning (9, 11, 16), it is likely that elevated lead exposures have a widespread effect on condor morbidity, based on the well-documented effects of lead on the peripheral and central nervous, renal, immune, reproductive, and hematopoietic systems in humans, laboratory animal models, and wildlife at blood lead levels as low as 100 ng/mL or lower (19–26). Revealing sources of lead exposure for the California Condor will aid in the remediation and prevention of elevated exposures to this endangered species and other wildlife.

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