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ORIGINAL ARTICLE

The frequency distribution of lead concentration in feathers, blood, bone, kidney and liver of golden eagles *Aquila chrysaetos*: insights into the modes of uptake

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Abstract Several cases of acute lead poisoning of golden eagles *Aquila chrysaetos* have been documented in the Alps. The question, however, remains how often golden eagles take up lead (once, chronically or episodically) and whether this uptake is in fatal or sublethal amounts. We approached this question by examining the level and frequency distribution of lead concentration in different tissues and in three segments of flight feathers in 41 golden eagles found dead, injured or moribund in the Swiss Alps. The frequency distribution of lead concentration in the blood, liver, kidney, wing coverts and shaft of flight feathers were all right-skewed. The highest values in blood, kidney and liver reached levels typical for acute fatal poisoning. In contrast, the frequency distribution of lead in bones was more symmetrical, but 71 % had bone lead concentrations >10 μg/g, which are considered elevated, and 29 % >20 μg/g, values often observed in cases of lethal poisoning. In 22 % of individuals, only one segment of a flight feather had a high lead concentration, while the other two segments had a low concentration. These findings indicate an episodic intake of lead of various amounts that may be immediately fatal (generating high blood levels) or sublethal. The patterns of lead in flight feathers and in bone suggest a repeated sublethal lead intake by the same individual. Such an episodic lead uptake seems only possible through ingestion of lead particles from carcasses or offal left behind by hunters. This also constitutes a risk to other scavengers, notably to the bearded vulture *Gypaetus barbatus* for which several high bone lead values have been found.

Keywords Golden eagle · Lead poisoning · Lead ammunition · Lead in feather

Zusammenfassung

Die Häufigkeitsverteilung von Bleikonzentrationen in Federn, Blut, Knochen, Niere und Leber von Steinadlern *Aquila chrysaetos*: Einblicke in die Art und Weise der Aufnahme

Mehrere Fälle von akuter Bleivergiftung von Steinadlern *Aquila chrysaetos* sind aus den Alpen bekannt. Die Frage ist, wie oft Steinadler Blei aufnehmen (einmal, chronisch oder episodisch) und ob dies in letalen oder subletalen Dosen geschieht. Wir untersuchten die Konzentrationen von Blei und ihre Häufigkeitsverteilungen in verschiedenen Geweben und in drei Abschnitten von Flugfedern von 41 Steinadlern, die tot, verletzt oder sterbend in den Schweizer Alpen gefunden wurden. Die Häufigkeitsverteilungen der Bleikonzentrationen in Blut, Leber, Niere, Flügeldeckfedern und dem Kiel von Flugfedern waren alle rechtsschief. Die höchsten Werte in Blut, Leber und Niere erreichten Werte, die typisch für letale Vergiftungen sind. Die Häufigkeitsverteilung der Bleikonzentrationen in Knochen

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hingegen war eher symmetrisch, wobei 71 % der Individuen Konzentrationen >10 µg/g, die als erhöht gelten, und 29 % Werte >20 µg/g zeigten, wie sie häufig bei letalen Vergiftungen auftreten. Bei 22 % der Individuen zeigte nur einer von drei Kielabschnitten einer Flugfeder erhöhte Bleiwerte, während die anderen beiden Abschnitte niedrige Werte aufwiesen. Diese Befunde legen nahe, dass die Aufnahme von Blei episodisch und in unterschiedlicher, unmittelbar tödlicher oder subletaler Menge erfolgt. Das Muster von Blei in Flugfedern und Knochen zeigt, dass die mehrmalige Aufnahme einer subletalen Menge Blei durch dasselbe Individuum wohl öfter vorkommt. Solch eine episodische Bleiaufnahme findet sehr wahrscheinlich über die Aufnahme von Bleipartikeln in Kadavern oder im Aufbruch von gejagten Tieren statt. Dies ist auch ein Risiko für andere Aasfresser, insbesondere für den Bartgeier *Gypaetus barbatus*, bei welchem mehrere sehr hohe Bleiwerte in Knochen gefunden wurden.

Introduction

Most lead is brought into the natural environment via human activities (Nriagu 1989). Leaded petrol resulted in a general contamination that has decreased or become insignificant since its ban. Other sources of lead, mostly restricted in space, are mining, waste dumps, industrial plants, paint chips, fireworks and sludge from sewage treatment facilities. Since feathers function as particle collectors in the airstream, measurement of lead and other heavy metals on feathers has been used as an indicator of general heavy metal pollution (Ellenberg et al. 1985; Hahn et al. 1989; Dmowski 1999).

A wide-spread source of lead is from ammunition for hunting, shooting exercises (shooting ranges, clay-pigeon shoots) and military exercises resulting in lead particles of various sizes (shot or bullet fragments) spread out in the field or in dead or live animals. Lead is a poison to wildlife affecting all body systems. The ingestion of only a single lead shot pellet may be lethal (Sanderson and Bellrose 1986). The lethal effect from the intake of lead shot pellets by waterfowl and waders has been extensively documented including the fact that lead poisoning resulted in about 1.6–2.4 million dead waterfowl annually in the USA (USFWS 1986). These findings finally resulted in a ban of lead shot for waterfowl hunting in many countries (Avery and Watson 2009).

However, also terrestrial birds have been recognized to be exposed to lead (Fisher et al. 2006; Pain et al. 2009; Franson and Pain 2011; Haig et al. 2014). Spent shot can be ingested directly from the ground (e.g., by terrestrial gamebirds such as pheasants and partridge) or from dead (i.e., unretrieved by hunters) or live (i.e., having survived shooting) prey by raptors. Conventional lead-based rifle bullets splinter into often very small, invisible fragments when penetrating a body (Scheuhammer et al. 1998). Because the incidence of lead particles retained by a large body is very high, mammal carcasses or their offal left behind by hunters in the field are prominent sources of lead to scavengers. For example, in the USA, >90 % of deer carcasses contained bullet fragments (Hunt et al. 2009), and in the province of Sondrio, Italy, >60 % of hunting offal of roe deer, red deer, chamois and wild boar contained lead fragments of ammunition bullets (Bassi and Ferloni 2012). In bald *Haliaeetus leucocephalus* and golden eagles *Aquila chrysaetos*, 10–15 % of post-fledging mortality has been attributed to direct poisoning by lead ingested from prey animals (Scheuhammer and Norris 1996; Helander et al. 2009). Another possible source of lead is non-fatal shot embedded in the body that is partly metabolized (Pain et al. 2009). In white-tailed sea eagles *Haliaeetus albicilla*, lead intoxications from feeding on live waterfowl with embedded shot and carcasses with remains of lead ammunition have been the most important cause of death (Krone et al. 2009).

In golden eagles, lead poisoning has been documented in North America (Craig et al. 1990; Kramer and Redig 1997; Wayland et al. 1999), the UK (Pain et al. 1995), Sweden (Kendall et al. 1996), Spain (Cerradelo et al. 1992) and the Alps (Austria, Switzerland and Germany; Bezzel and Fünfstück 1995; Zechner et al. 2005; Kenntner et al. 2007; Madry et al. 2015). In all cases, ingestion of spent lead ammunition was found or suspected to be the reason for poisoning.

The question, however, remains how often the population of golden eagles in the Swiss Alps takes up lead and whether this is in fatal or sublethal amounts. Golden eagles (and possibly other avian or mammalian scavengers) may be exposed to a continuous chronic lead supply (e.g., from the environment via the food chain or, likely to a lesser degree, from lead pellets embedded in their body), may suffer from repeated episodic ingestion of sublethal amounts of lead (e.g., lead particles from several carcasses ingested over time) or may be exposed to a single lead ingestion resulting in an acute and possibly fatal lead poisoning. Sublethal chronic lead assimilation may result in higher mortality or reduced reproduction (Pain et al. 2009), potentially affecting a much higher proportion of the population than evidenced from individuals found with signs of acute lead poisoning.

This question can be approached by examining the level and frequency distribution of lead concentrations in different tissues. Lead in blood increases within 24 h of lead particle ingestion (Hoffman et al. 1981) and has a half-life of about 2 weeks (Pain et al. 2009; Fry et al. 2009). This

means that lead from particles regurgitated hours or days later may be partly absorbed. Lead is then transported by the bloodstream to organs and bones. Liver and kidney retain lead compounds for a few days to months after a distinct lead intake (Fisher et al. 2006; Pain et al. 2009). In bones lead appears rapidly after lead ingestion (Sanderson and Bellrose 1986) and is accumulated; judging from a turnover of 10,000 days in humans, bone lead reflects lifetime exposure in wildlife (Fisher et al. 2006). In and on feathers, lead is deposited in two ways: internally through incorporation via the bloodstream during feather growth and externally through contamination of the complex feather surface from air pollution, preening and contact with dust, soil and water (Dauwe et al. 2003). However, the rachis is hardly contaminated externally, while the barbs can be contaminated heavily with external lead (Cardiel et al. 2011). Since feathers grow at an approximately constant rate and are metabolically inactive after formation, inferences on the time pattern of lead ingestion can be made if the lead content is analyzed in different segments of the rachis of large feathers.

Hence, the pattern of occurrence of lead in different tissues and along feathers can give valuable information about the sources of lead, amount and time frame of lead uptake (recent, acute, chronic, episodic).

The aim of this article is to use the frequency distributions of lead concentration in several tissues of golden eagles from Switzerland to derive how lead was incorporated. This is a companion paper to the article by Madry et al. (2015), which compares lead contamination of golden eagles with that of eagle owls *Bubo bubo* and the prey of both species.

Materials and methods

Sample collection

In total 41 golden eagles found dead, injured or moribund in the Swiss Alps were collected by gamekeepers of the Canton of Grisons (31 individuals) and by various people from other cantons of Switzerland (Berne 3 individuals, Glarus 2, Lucerne 2, St. Gallen 2, Ticino 1) from 2006 to 2013. The majority (22 individuals) were casualties of fatal intraspecific fights, 6 individuals showed signs of acute lead poisoning (paralysis, apathetic appearance, thin green excrements), 1 of barbiturate poisoning (vomiting), 1 juvenile left the nest too early, 1 individual was electrocuted, and for 10 individuals reasons of death were unknown.

Most birds were autopsied at the Fish and Game Department of the Canton Grisons, additional birds at the Veterinary Universities of Zurich and Berne. Age was determined from the pattern of wing feather moult and sex from reproductive organs. We X-rayed 28 birds to determine whether they were shot or had ingested shot or bullet fragments. Sampling of tissues depended on whether the bird was dead or was alive and could be rehabilitated and released (hence no samples from bones, liver and kidney) and on local circumstances. Therefore, sample size varied between tissue samples (see Table 1 for sample sizes).

From secondary feathers the vanes were removed as they are prone to accumulate lead from environmental contamination (Dmowski 1999; Cardiel et al. 2011). From the underwing, one or two whole coverts were taken. Bone samples were taken from the sternum (7 individuals), femur (8), ulna (1) or pelvis (1). Lead seems to accumulate in different bones to similar levels, although differences may exist at low concentrations (Mateo et al. 2003; Ethier et al. 2007). Feather samples were stored at room temperature in plastic bags in light-inhibiting boxes. Viscera and bone samples were frozen in plastic boxes for storage at −20 °C. Blood was stored with EDTA in tubes at 4 °C.

Determination of lead concentration

The feather shaft of the secondary flight feathers was cut with a stainless steel scalpel into three segments according to Rodriguez-Ramos Fernandez et al. (2011): segment A representing the calamus, segment B and C representing the proximal and distal part of the rachis. All feather samples were decontaminated similarly to the method reported in the literature with deionized water (18.2 MΩ cm,

Table 1 Sample sizes, median concentration, skewness and kurtosis of the frequency distribution of lead concentration in blood, liver, kidney, bone, wing coverts and flight feather shaft

	Lead concentration (μg/g) in					
	Liver	Kidney	Flight feather shaft	Wing covert	Blood (μg/dl)	Bone
No. of individuals	26	25	21	11	7	17
Median	1.16	0.99	0.22	0.38	6.60	12.45
Skewness (±SE)	4.97 (±0.46)	4.81 (±0.46)	2.70 (±0.50)	2.60 (±0.66)	1.53 (±0.79)	0.81 (±0.55)
Kurtosis (±SE)	25.05 (±0.89)	23.67 (±0.90)	8.37 (±0.97)	7.22 (±1.28)	1.85 (±1.59)	−0.40 (±1.06)

The tissues are ordered from high to low values of skewness and kurtosis

Purelab® Ultra Water system), acetone and 2 % nitric acid by shaking for 2 min with each solution followed by rinsing twice with deionized water (Rodriguez-Ramos Fernandez et al. 2011). Wing coverts were analyzed in total.

After thawing, livers and kidneys were weighed and dried to constant weight at 100 °C. Duplicate samples (average weight 150 mg) were analyzed. After thawing the bones, the adherent tissue was removed with a stainless steel scalpel and dried in an oven for 4 h. Duplicate samples (average weight 250 mg) were analyzed. Blood samples were thawed and diluted with deionized water in a 1:10 ratio for analysis.

All samples were digested in closed Teflon® vessels using 4 ml of 65 % (v/v) nitric acid (Suprapur, Merck) and 0.5 ml of 30 % (v/v) hydrogen peroxide (Suprapur, Merck). Digestion was performed by a Microwave Digestion System (UltraCLAVE, Milestone Srl). The heating program was operated in three steps: $t = 0$–10 min: temperature (T) increase to 220 °C; $t = 10$–14 min: T increase from 220 to 250 °C; $t = 14$–24 min: T at 250 °C; pressure and energy were 160 bars and 1000 W, respectively, throughout the run. The digestion method was followed by cooling for 1.25 h. After decomposition the samples were transferred to plastic flasks and stocked to 50 ml with deionized water followed by a dilution in a ratio of 1–5. One milliliter was used for analysis.

Lead concentration was determined via an inductively coupled plasma mass spectrometer (ICP-MS) by Varian (Darmstadt, Germany). Calibration curves were prepared in aqueous solutions using ICP Multi Element Standard Solution XXI CertiPur (Merck). For quantification the average of lead isotopes ^{206}Pb, ^{207}Pb and ^{208}Pb was used. Two different positive control samples were used to verify the accuracy of the analytical method: Human Hair Certified Reference Material No. 13 (National Institute for Environmental Studies, Japan), with a certified lead concentration of 4.6 ± 0.6 µg/g, and SeronormTM Trace Elements Whole Blood at lead concentration levels of 14.8 ± 1.0 µg/l and 336 ± 36 µg/l. Negative control samples were prepared in the same manner as the samples but without material. The limit of quantification for lead was 0.1 µg/l. Lead concentrations are based on a dry weight basis (µg/g) except for blood.

Data analysis

The shape of the frequency distributions of lead concentration in various tissues was characterized with two measures (in SPSS 18). Skewness indicates the degree to which a frequency distribution has a longer or fatter tail toward the right than left end (positive values; skewed to the right) or vice versa (negative values; skewed to the left) (Sokal and Rohlf 1995). Kurtosis indicates the degree to which a frequency distribution is peaked more than a normal distribution (positive values; leptokurtic) or less (negative values; platykurtic) (Sokal and Rohlf 1995).

Results

The frequency distribution of lead concentration in wing coverts, the shaft of secondary flight feathers, blood, liver, kidney and bone showed considerable differences in median values and shape, expressed as skewness and kurtosis (Fig. 1; Table 1). Lead concentration in liver and kidney showed a frequency distribution highly skewed to the right and leptokurtic (peaked). In fact, the values of one individual were very high (77.4 µg/g in liver and 30.9 µg/g in kidney), indicating an acute poisoning, while another individual had an elevated liver value (8.4 µg/g). Liver and kidney lead levels in golden eagles <6 µg/g dry weight indicate exposure to background lead concentrations. Levels >6 µg/g are considered elevated; levels >30 µg/g in the liver and >20 µg/g in the kidney represent lethal poisonings (Wayland et al. 1999; Clark and Scheuhammer 2003; Madry et al. 2015).

The distribution of lead concentration in wing coverts, the shaft of flight feathers and blood was also right-skewed and leptokurtic, but to a lesser degree than in liver and kidney (Table 1; Fig. 1). Three of 11 individuals (27 %) had lead levels in wing coverts higher than 1 µg/g and 1 out of 21 individuals (5 %) in the shaft (means of all 3 segments). The highest value was 5.0 µg/g in a wing covert. The three individuals with a high lead concentration in blood (32.0, 56.3, 108.0 µg/dl) all showed signs of acute lead poisoning. Blood concentrations <20 µg/dl are considered normal; levels >50 µg/dl represent poisoning (Franson et al. 1983; Garcia-Fernandez et al. 1997; Pattee et al. 2006; Stansley and Murphy 2011; Harmata and Restani 2013).

The distribution of lead concentration in bone was more symmetric and platykurtic. Twelve out of 17 individuals (71 %) had bone lead concentrations >10 µg/g, which are considered elevated, and 5 of them (29 %) >20 µg/g, values often observed in lethal poisoning (Mateo et al. 2003; Rodriguez-Ramos Fernandez et al. 2011).

Lead concentrations in liver and kidney were highly positively correlated ($r = 0.99$, $p < 0.001$, $n = 25$). To a lesser degree, the lead concentration in bone was correlated with that in both liver and kidney ($r = 0.66$, $p = 0.02$, $n = 12$; $r = 0.85$, $p < 0.001$, $n = 12$). Lead in flight feather shaft (mean per individual) was not significantly correlated with liver ($r = 0.09$, $p = 0.78$, $n = 13$), kidney ($r = -0.01$, $p = 0.97$, $n = 12$) or bone values ($r = -0.20$, $p = 0.52$, $n = 13$). The sample size was too small (<6) to examine the remaining correlations among tissues.

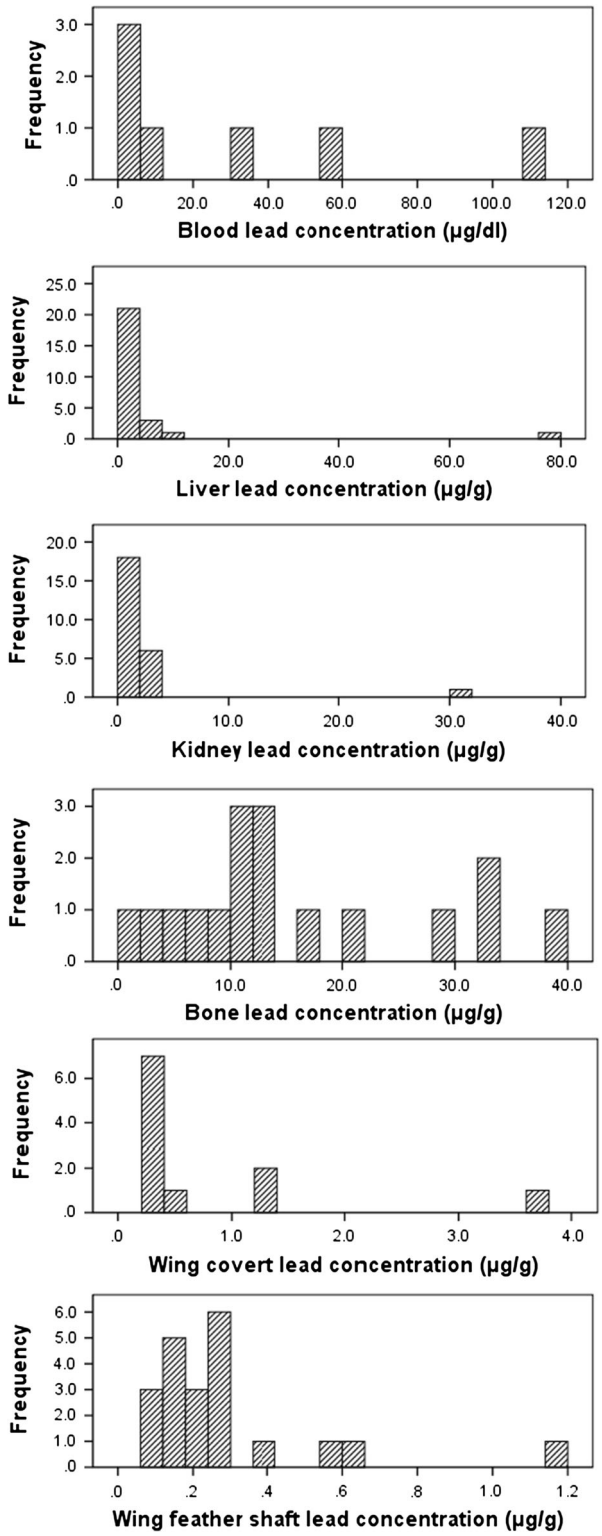

Fig. 1 Frequency distributions of lead concentration in the blood, liver, kidney, bone, wing coverts and shaft of flight feathers. The mean was taken of shaft segments and if several values per individuals were available. All concentrations are per dry weight (µg/g), except for blood (µg/dl). For sample sizes, see Table 1. Note that the scale of the *x* axis varies between tissues

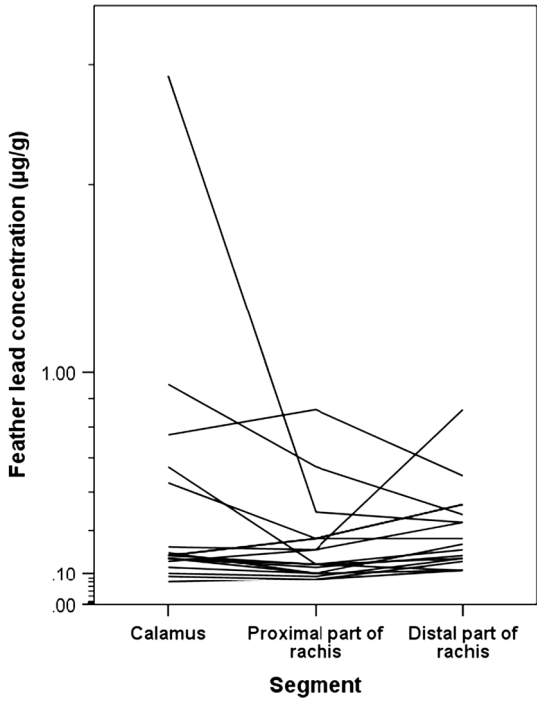

Fig. 2 Lead concentration in segments of the shaft of individual flight feathers ($n = 18$). Each flight feather is from a different individual golden eagle. Note the logarithmic *y* axis

In 13 of 18 flight feathers, the lead concentration was low (<0.5 µg/g) in all three segments of the shaft, while in one individual all segments showed high values (>0.5 µg/g). In four individuals (22 %), only one segment had a high lead concentration, while the other two had a low concentration (Fig. 2); the segment with a high concentration was the calamus or the distal part of the rachis.

Discussion

In this study we showed that the frequency distributions of lead concentration in the blood, liver, kidney, wing coverts and shaft of flight feathers of a sample of golden eagles found dead or moribund were all right-skewed with the highest values reaching elevated levels or even levels typical for acute poisoning. In contrast, the frequency distribution of lead in bones was hardly right-skewed, but still some values reached high levels. From this, the following insights on the temporal pattern and amount of lead uptake can be gained.

Blood lead values were right-skewed, similar to those in Egyptian vultures *Neophron percnopterus* and four species of Spanish raptors (Garcia-Fernandez et al. 1997; Gangoso et al. 2009). The three highest values were from individuals showing acute symptoms of lead poisoning (Fig. 1), which

were given therapy and released. This confirms that acute lead poisoning occurs in golden eagles in the Swiss Alps. They would probably have died if left unattended. Thus birds having undergone a heavy acute lead poisoning will most probably be absent from the sample of feathers and bones collected from live birds without acute symptoms (e.g., Pattee et al. 1981).

Hepatic and renal lead concentrations were strongly right-skewed, with one individual showing values of heavy poisoning. This is similar to the right-skewed distributions of liver and kidney lead in white-tailed eagles *Haliaeetus albicilla* from Germany (Kenntner et al. 2001) and the USA (Wayland et al. 1999). The strong correlation between liver and kidney, as observed in many studies (see Wayland et al. 1999), indicates a systemic lead load. The extreme right-skewness with values only rarely reaching very high values may indicate that birds rarely survive a lead intoxication reaching these vital organs, but may die before, i.e., at the stage of very high blood levels.

In bones, the lead concentration was more symmetrically distributed, as also found in eagles from the US (Wayland et al. 1999), but unlike the right-skewed distributions in various raptor species in Spain (Mateo et al. 2003) and in Spanish imperial eagles *Aquila adalberti* (Pain et al. 2005). The lead values found in bones in this study are among the highest found in golden eagles (Wayland et al. 1999; Clark and Scheuhammer 2003; Mateo et al. 2003) and clearly indicate a substantial sublethal contamination (Madry et al. 2015). As shown with the isotope signature of lead in soil, ammunition, prey animals of golden eagles and bones of golden eagles, lead in bones of golden eagles is most probably from the ingestion of metallic lead embedded in shot animals or their offal (Madry et al. 2015). High bone lead concentrations may also indicate a recent lead intake, because renal and hepatic lead correlated to some extent with bone lead, as also found in several other studies (Wayland et al. 1999; reviewed in Mateo et al. 2003). Lead is said to appear in bones relatively rapidly after lead intake (Sanderson and Bellrose 1986) and is retained a long time or life-long. If not mobilized, lead in bones is thus much less toxic than lead in organs. This may explain the absence of a right-skewed distribution as compared to the soft tissues.

The lead concentration in feathers was also right-skewed to some degree, as found for example in seabirds (Burger and Gochfeld 2000b) and Spanish imperial eagles (Pain et al. 2005). Because lead in the feather shaft is hardly contaminated with external lead (Cardiel et al. 2011), this indicates that some individuals have been exposed to elevated lead uptake during the time of feather growth. Because the feather lead concentration was not correlated with that of other tissue and the feathers examined were not growing, the events of lead intake during feather growth and that of other organs were clearly temporally separated. The quite unequal lead concentrations in parts of the shaft of the same feather in four specimens, as also found in the Spanish imperial eagle (Rodriguez-Ramos Fernandez et al. 2011), indicate that lead uptake is clearly temporally restricted to a few days or weeks, the time needed to grow that particular part of the feather (a golden eagle wing feather grows at a rate of about 7 mm/day; S. Denis personal communication and own observations). Golden eagles moult between March/April and September (Cramp 1980), but growing flight feathers are also observed until late autumn and even in winter (Bloom and Clark 2001, own observations). Hence, golden eagles may incorporate lead over much of the year including the autumn hunting season.

In summary, the patterns of frequency distributions and elevated, sometimes high concentrations of lead found in golden eagles from Switzerland suggest an episodic intake of lead of various amounts that may be immediately fatal (high blood levels) or sublethal. The pattern of lead in flight feathers and bone suggests that repeated sublethal lead uptake by the same individual is probably not infrequent. Such an episodic lead uptake is likely through ingestion of lead particles from carcasses or offal in the case of golden eagles in the Swiss Alps (Madry et al. 2015). Lead from air pollution could still be available to animals (see, e.g., Scheifler et al. 2006), but this is unlikely to be the case in the thinly industrialised Alps and would result in a chronic intake of low levels. Similarly, bioaccumulation of lead via the food chain would also result in a continuous chronic uptake of low levels (see Madry et al. 2015). Lead shot embedded in a golden eagle was found in only one case. This bird showed elevated lead values in the bones (10.5 μg/g), but not in the feathers. The isotope signature of lead in golden eagles was very similar to that of the ammunition used in this region and unlike the isotope signature found in soil (also from old mining areas) and bones of prey animals (Madry et al. 2015).

The amount of lead uptake by the organism depends on the amount of lead ingested and the residence time in the alimentary tract. Lead may be ingested as very small (invisible) fragments or in high amounts (up to 40 shot pellets in the proventriculus of a golden eagle in Spain; Cerradelo et al. 1992). Elemental lead fragments may be regurgitated by golden eagles in pellets after some time. However, the gastric juice of raptors has a particularly low pH (Redig and Arent 2008; Rodriguez-Ramos Fernandez et al. 2011), and dissolution and uptake of lead is enhanced by low pH in portions of the digestive tract (Rattner et al. 2008). Hence, even a short gastric residence time of a few hours may dissolve some lead. Therefore, depending on the residence time in the body, the amount of lead uptake may vary greatly even when the amount ingested is the same.

Hence, high amounts of lead intake need not be fatal. This may explain why golden eagles have been found to show signs of repeated, episodic sublethal lead uptake.

The examination of several tissues of golden eagles revealed acute strong lead poisoning as well as repeated sublethal uptake. While many of the acute lead poisoning instances are probably fatal in the wild, the question is what effects repeated sublethal uptake may have. It is well-known that lead, even in low doses, has deleterious effects on all body systems. Lead at sublethal levels has negative effects on cognition and behaviour, physiology, immunity, hemoglobin production, bone mineralization and reproduction in birds (e.g., Burger and Gochfeld 2000a; Redig et al. 1991; Gangoso et al. 2009; reviewed in Pain et al. 2009).

It remains to be examined whether sublethal lead contamination in golden eagles in Switzerland has any impacts on reproduction and survival despite the fact that the population of golden eagles in the study area is stable or increasing (Haller 1996). Whenever lead ammunition is used, all predatory and scavenging birds and mammals are potentially at risk from lead poisoning. This concerns mainly scavengers feeding on shot animals, offal left in the field by hunters and animals that have been shot at but survived, carrying lead in their body. Studies on the California Condor *Gymnogyps californianus* showed that mortality from ingested ammunition lead was a major cause of the decline and virtual extinction of the species and limits the re-establishment of a viable population from released birds (Meretsky et al. 2000). In the Swiss Alps, the recently re-introduced bearded vulture *Gypaetus barbatus* may be particularly at risk, although this small population is increasing and reproducing well (Schaub et al. 2009). Indeed, among four bearded vultures from the Alps, only one had a low lead concentration in bone (6.50 μg/g), while three had very high values (38.90–100.04 μg/g; Bassi et al. 2013, in press; own unpublished data).

From this study it appears that lead particles from carcasses or offal killed by shot or bullets may pose a risk to golden eagles and other scavengers in the Swiss Alps (see also Madry et al. 2015). We advocate the examination of several tissues for lead to reveal acute, recent or past lead uptake. The examination of several feathers of the same individual grown at different times may be particularly rewarding to reveal the frequency and time course of episodic lead uptake over a longer time. Flight feathers in adult golden eagles are replaced sequentially every 2–3 years and thus can provide an archive of lead intake over a few years. Moreover, a systematic study on the effects of sublethal lead contamination on reproduction and survival is needed. However, a more elegant solution would be to replace all lead ammunition by lead-free ammunition (e.g., Trinogga et al. 2013).

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