Environmental Science & Technology

Article

pubs.acs.org/est

Sublethal Lead Exposure Alters Movement Behavior in Free-Ranging Golden Eagles

Frauke Ecke,[*,†,‡,∞] Navinder J. Singh,[†,∞] Jon M. Arnemo,[†,§] Anders Bignert,[∥,⊥] Björn Helander,[∥] Åsa M. M. Berglund,[#] Hans Borg,[∇,Å] Caroline Bröjer,[○] Karin Holm,[∇] Michael Lanzone,[◆] Tricia Miller,[¶] Åke Nordström,[†] Jannikke Räikkönen,[∥] Ilia Rodushkin,[⊗] Erik Ågren,[○] and Birger Hörnfeldt[†]

[†]Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, SE-90183 Umeå, Sweden

[‡]Department of Aquatic Science and Assessment, Swedish University of Agricultural Sciences, Box 7050, SE-75007 Uppsala, Sweden

[§]Department of Forestry and Wildlife Management, Inland Norway University of Applied Sciences, Campus Evenstad, NO-2480 Koppang, Norway

[∥]Department of Environmental Research and Monitoring, Swedish Museum of Natural History, Box 50007, SE-10405 Stockholm, Sweden

[⊥]State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China

[#]Department of Ecology and Environmental Science, Umeå University, SE-90187 Umeå, Sweden

[∇]Department of Environmental Science and Analytical Chemistry, ACES, Stockholm University, SE-10691 Stockholm, Sweden

[○]Department of Pathology and Wildlife Diseases, National Veterinary Institute (SVA), SE-75189 Uppsala, Sweden

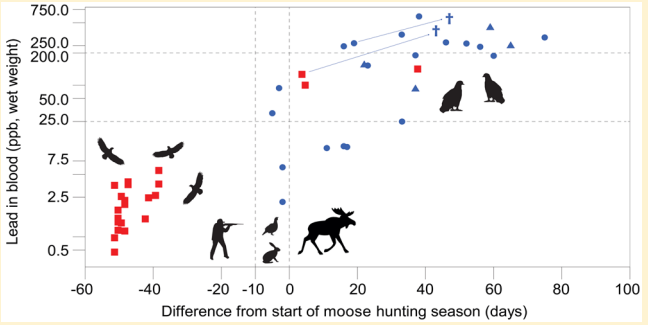
[◆]Cellular Tracking Technologies, Rio Grande, New Jersey 08242, United States

[¶]Division of Forestry and Natural Resources, West Virginia University, Morgantown, West Virginia 26506, United States

[⊗]ALS Scandinavia AB, Aurorum 10, SE-97775 Luleå, Sweden

Supporting Information

ABSTRACT: Lead poisoning of animals due to ingestion of fragments from lead-based ammunition in carcasses and offal of shot wildlife is acknowledged globally and raises great concerns about potential behavioral effects leading to increased mortality risks. Lead levels in blood were correlated with progress of the moose hunting season. Based on analyses of tracking data, we found that even sublethal lead concentrations in blood (25 ppb, wet weight), can likely negatively affect movement behavior (flight height and movement rate) of free-ranging scavenging Golden Eagles (*Aquila chrysaetos*). Lead levels in liver of recovered post-mortem analyzed eagles suggested that sublethal exposure increases the risk of mortality in eagles. Such adverse effects on animals are probably common worldwide and across species, where game hunting with lead-based ammunition is widespread. Our study highlights lead exposure as a considerably more serious threat to wildlife conservation than previously realized and suggests implementation of bans of lead ammunition for hunting.



INTRODUCTION

Predators and scavengers are important for ecosystem functioning and ecosystem health by removing carcasses and wounded or weak animals.[1] Lead poisoning of predators and scavengers from ingestion of fragments of lead-based ammunition in carcasses and offal of shot wildlife is well acknowledged globally[2−6] and is potentially causing sublethal or even acute lead poisoning in these groups. Several sublethal effects of lead poisoning in wildlife have been suggested, including behavioral changes.[2,7] However, such behavioral changes have never been documented and quantified in free-ranging animals.

A number of factors are known to affect movement and behavior in free ranging animals.[8,9] These include internal factors such as animal life history (age and sex), physiology, body condition and state, as well as external factors such as climate, human land use, pollution, food, predation, competition, and disease.[10−13] The relative importance of these factors may also vary at different scales of movement which may

Received: November 29, 2016
Revised: April 10, 2017
Accepted: April 17, 2017
Published: April 17, 2017

DOI: 10.1021/acs.est.6b06024
Environ. Sci. Technol. 2017, 51, 5729−5736

further influence species population demography and dynamics.[8]

Today, lead ammunition used for big game hunting is the main source of lead poisoning in scavenging birds.[2−6] This is evident from comparisons between lead concentrations during and outside the hunting season, as well as from an increase in hunting pressure,[4,14] and from lead isotopic analysis.[5] Exposure may occur from consumption of offal of hunter shot game, from mortally wounded, but unrecovered game, or game that are wounded and not recovered and die at some later date. This not only applies to big game, but also small game; for example, high percentages of Willow Ptarmigans (*Lagopus lagopus* L.)[15] and mountain hares (*Lepus timidus* L.)[16] survive hunting with lead shot embedded in their tissues, and birds preying or scavenging on wounded small game may be exposed to this lead.

Clinical signs of lead-poisoned birds include anorexia, anemia, neurological effects, and wing droop.[17] Experimental, sublethal lead concentrations in gull and tern chicks affected locomotion, balance and depth perception.[18] Sublethal concentrations of lead are suggested to increase risk of collision.[6,7] This is likely related to impaired flight performance (and ability to avoid danger), which could also cause reduced hunting success with subsequent starvation. For scavengers, thresholds identified in *Falconiformes* are currently recommended in ecotoxicological studies[5] with background concentrations being lower than subclinical lead concentrations that range between 200 and 500 ppb lead in blood and between 2000 and 6000 ppb ww (wet weight) in liver,[17] which is similar to the subclinical range proposed in Bald Eagles (*Haliaeetus leucocephalus* L.).[19] The hitherto assumed thresholds for toxic lead concentrations are mainly nonspecies specific, often based on simplified experiments on birds in standardized captivity[18] (but see Hoffman et al.[20] for lead-induced effects on hematological parameters in captive Bald Eagles), and primarily based on physiological effects.[20−22] Eagle-specific diagnostic blood and/or liver thresholds for lead have been reported previously[19,23,24] but to our knowledge no eagle-specific behavior-based thresholds for lead concentrations have been identified.

Advancements in Global Positioning System (GPS) and other emerging technologies for tracking animals have rapidly increased our ability to study and quantify movement behavior of free living animals, thereby allowing us to reveal intricate details about various aspects of species life history and cycle, habitat selection, flight behavior and demographic consequences of movement.[25,26] These advancements now also provide opportunities to study the effects of environmental contaminants on altering animal behavior, which has not been previously possible.[27]

The Golden Eagle (*Aquila chrysaetos* L.) is rare, long-lived and widely distributed across the northern hemisphere.[28] It is listed in Annex 1 (species needing special habitat conservation measures) of the EU Birds Directive.[29] Most Golden Eagles in Sweden breed in the country's northern boreal forest from ca. 60°N to 66°N latitude, relying on old trees for nesting and open areas like clear-cuts for hunting.[30,31] Their diet varies with season with grouse species and mountain hare dominating the diet in the breeding season.[32] Knowledge of diet outside the breeding season is very limited, but scavenging appears to be important.[28] Golden Eagles in Sweden undertake age-dependent partial migrations, whereby juvenile birds migrate south in late autumn (late September to October) and return to north during spring (late April to early May).[33,34] Home range size also varies between breeding and nonbreeding period, where range size is larger during the nonbreeding season than during the breeding season.[31,34] Flight altitudes of eagles are variable throughout the year and depend upon habitat type. Eagles fly highest above water and urbanized areas. Flight altitudes above other habitat types such as forests, mires, and pastures are highly variable.[34]

To further our knowledge of the effects of lead on free-ranging animals, we studied flight performance in scavenging Golden Eagles from which we had information on blood lead levels. We also compared liver lead levels to causes of death of Golden Eagles from specimen banks. We predicted that high lead concentrations in blood would affect flight height and movement rates of tracked eagles. This should also be reflected in high lead concentrations in recovered eagles that died from collisions or that died due to causes that might be related to reduced hunting skills (e.g., starvation) or alertness (e.g., poaching) of the eagles.

MATERIALS AND METHODS

Golden Eagle Trapping, Blood Sampling and Tagging. Forty-six Golden Eagles were captured in the four northernmost counties in Sweden (Västernorrland, Jämtland, Västerbotten, and Norrbotten county) and equipped with backpack type CTT (Cellular Tracking Technologies Inc., 70 g) GSM (Groupe Spécial Mobile) transmitters in 2014 and 2015. The 23 juveniles were young of the year, four were subadults in their second (2K) to fifth calendar year (5K); three were aged from rings (one 2K and two 5K) and one estimated from plumage (tail pattern) to 3K and the 19 adults were 6K birds or older aged from rings and/or tail patterns (see Tjernberg and Landgren[35] for description of age classification). Gender was determined from weight and wing length measurements. Juveniles in 2014 were captured in the nest near to fledging age,[36] while adults, subadults and juveniles in 2015 were trapped with bow nets[37] in the postfledging season in summer-autumn (late August−November) in open areas such as clear-cuts within their territories. A volume of 1−2 mL blood was taken from the brachial wing vein of each eagle. Care was taken to ensure that marked birds were in good condition before and after sampling (bright looking plumage, no drooping eyelids, no visual signs of disease and no drooping wings) and that all birds were able to fly after their release (with the exception of juveniles in the nest).

Permissions for the study were obtained from the Swedish Environmental Protection Agency, Stockhom (Dnr NV-04092-14) and the Swedish Ethical Committee on Animal Research, Umeå (Dnr A40-14 and A42-14).

Data Set on Dead Golden Eagles. In Sweden, dead Golden Eagles belong to the state and must be handed in to the authorities (Swedish Museum of Natural History or Swedish National Veterinary Institute; SVA) for post-mortem investigations and banking. A total of 111 eagle livers banked and stored at −20 or −25 °C during 2003−2011 were available for study. Assessments on cause of death were based on evidence from field-site circumstances and observations from necropsies conducted by SVA. Our main interest was to compare subclinical concentrations of lead among categories of cause of death. Among the 111 specimens, eight were considered lethally lead poisoned[17] with liver lead concentrations in the range 8600−56 900 ppb lead ww. These eight specimens were excluded from the analysis since we were interested in the

DOI: 10.1021/acs.est.6b06024
Environ. Sci. Technol. 2017, 51, 5729−5736

Table 1. Information on the 16 Eagles (Sorted by Year, Month and Day of Tagging) Analyzed for Flight Performance (Flight Height and Movement Rate), Including Their Weight, Gender (F, Female or M, Male), Date of Tagging, Lead Concentration in Blood at Date of Tagging, Number (n) of GPS-Positions Recorded and Analyzed During the 14-Day Study Period As Well As Mean Flight Height and Movement Rate (SD, Standard Deviation and CI, 95% Confidence Interval)

						flight height (m)			movement rate (km 15 min^{-1})		
eagle id	weight (g)	gender	date (YYYYMMDD)	lead concentration (ppb ww)	n	mean	SD	CI	mean	SD	CI
1	4730	F	20140830	2.17	696	74.07	158.97	11.83	0.70	1.40	0.10
2	4000	M	20150901	31.95	664	28.13	57.30	4.37	0.30	0.79	0.06
3	5250	F	20150903	68.70	674	45.45	91.36	6.91	0.58	1.28	0.10
4	3560	M	20140912	11.10	577	26.82	34.76	2.84	0.31	0.90	0.07
5	5435	F	20140917	11.80	560	38.30	69.23	5.75	0.67	1.70	0.14
6	5080	F	20140918	11.50	524	61.40	99.98	8.58	1.10	2.20	0.19
7	3850	M	20150922	244.00	577	27.07	36.74	3.00	0.46	1.01	0.08
8		M	20150925	267.00	557	21.98	56.59	4.71	0.23	0.67	0.06
9	4110	M	20150929	136.00	545	33.96	58.48	4.92	0.53	1.07	0.09
10	5500	F	20151009	346.00	432	22.95	19.22	1.82	0.24	0.69	0.06
11	5900	F	20151013	186.00	415	23.21	18.49	1.78	0.35	0.76	0.07
12	5300	F	20151014	600.00	406	25.83	17.43	1.70	0.29	0.69	0.07
13	4300	M	20151022	276.00	396	21.42	14.47	1.43	0.32	0.70	0.07
14	4200	M	20151028	265.00	355	26.82	20.02	2.09	0.41	0.91	0.09
15	5600	F	20151101	241.00	318	18.36	13.32	1.47	0.06	0.17	0.02
16	4250	M	20151120	320.00	3	22.48	8.17	20.31	0.05	0.06	0.16

effects of sublethal lead concentrations. We identified 10 categories of cause of death of which eight were included in the further visualization of data. The other two categories of death, collision with a wind turbine (3419 ppb lead ww in liver) and infection (1826 ppb lead ww in liver) were not included in the visualization because there was only one bird in each category. Twelve specimens in the sample were diagnosed with starvation/emaciation and their liver samples contained on average 28% dry matter, compared to 30% in the rest of the sample. This difference was considered negligible for comparisons of concentrations among categories of dead birds.

Identifying Lead-Caused Thresholds in Movement Behavior. There are several approaches for identifying thresholds in doses that affect animal behavior. The Benchmark dose approach (BMD)[38] overcomes several limitations associated with the no-observed-adverse-effect-level (NOAEL) approach. The BMD is determined as the dose corresponding to a predetermined change in the response, referred to as the benchmark response (BMR). Studies comparing levels of NOAEL and BMR values for several data sets suggest that the risk at NOAEL corresponds to a BMR level in the range of 5−10% or more than 10%.[39,40] The U.S. Environmental Protection Agency and European Food Safety Authority have suggested a BMR of 5 or 10%.[41−43] We fitted a sigmoid function to the dose−response data explaining a significant part of the variation in response, due to variation in concentration. We used the following function (eq 1):

$$y = y_0 + [(h - y_0)/(1 + x^n/c^n)] \quad (1)$$

.

The predetermined change was chosen to be 10% from the average flight height and movement rate, respectively, at very low lead concentrations (h); y_0 = average flight height and movement rate, respectively, at the highest observed lead concentrations; c and n are constants describing the inflection point and the steepness of the sigmoid, respectively; and x = lead concentration (ppb). The fitted sigmoid function was based on average values for both x and y values of at least two (flight height) or three (movement rate) observations for each of the average values. To avoid splitting observations that are very close, observations closer than 0.05% of the x-range, were allocated to the same average. It has to be stressed that two or three observations constitute a very small sample associated with huge confidence intervals and uncertainty. The x-values were sorted in increasing order. For flight height, the first two observations formed the first average; the second average was formed by the following two observations and so on. For movement rate the averages were based on three observations, except for the last average that was based on four observations since the last single observation could not form a new average.

In the analyses, we combined adult males (n = 8) and females (n = 8) since they showed similar lead blood concentration at date of tagging (Mann−Whitney U-test, U = 26, P = 0.56) and flight performance (flight height: U = 23, P = 0.37, movement rate: U = 22, P = 0.32).

Chemical Analyses. Preparation and analysis of blood and liver samples followed standard procedures and are described in detail in the Supporting Information (S1).

Data Analyses. The Golden Eagle movement data set is acquired, stored and handled using the Wireless Remote Animal Monitoring (WRAM) system[44] under the Swedish Golden Eagle Project. The data set for this study was downloaded from WRAM and used for statistical analyses of flight and movement parameters.

GPS locations (at maximum 15 min intervals) for 14 days postcapture were available and extracted for 16 of the 19 adult eagles to investigate flight heights and movement rate (km 15 min^{-1}) (Table 1). Our selected frequency of locations is reasonable for this kind of analyses, even though higher resolution data are starting to emerge from telemetry studies.[45,46] Three eagles were excluded from the analyses because they did not meet our selection criteria (i.e., they lacked position data during the study period 1 September to 31 December 2014 and 2015, respectively). Because we were interested in flight behavior, we only included positions recorded between dawn and dusk when eagles were flying.

DOI: 10.1021/acs.est.6b06024
Environ. Sci. Technol. 2017, 51, 5729−5736

We excluded all locations when eagles were perched or roosting. The GPS transmitter on one of these 16 eagles only recorded three locations at 15 min frequency during the study period. Nevertheless, we included this eagle in our analyses because of the available lead concentration in blood (320 ppb lead ww). The vast majority of juveniles were sampled for blood lead more than a month prior to the moose (*Alces alces* L.) hunting season, which is from the first Monday in September to mid-January each year. To obtain as large and homogeneous sample as possible, only adults were used in the movement analyses (Table 1).

Flight height (above ground level, AGL) was estimated by subtracting ground elevation extracted from a 5-m resolution Digital Terrain Model (acquired from the Swedish National Land Survey, *Lantmäteriet*) from GPS recorded height.

The vertical accuracy of CTT transmitters that we used in this study is rated at a vertical error probability (VEP) with 50% of the locations falling within <4 m vicinity of the actual location and two-dimensional (or distance) root-mean-square (2 dRMS) with 95% probability <6 m. We used 2 dRMS, as VEP was too low. The actual error for each fix is recorded by the GPS as the vertical dilution of precision (VDOP). To calculate the altitude error, we multiplied VDOP for each fix by the vertical accuracy. We then calculated the mean for each bird and averaged those to determine the grand mean (±SE) for all birds.[11,47] The horizontal precision was less than or equal to 2.5 m, and vertical precision was less than or equal to 22.2 ± 5.6 m. We converted eagle positions into linear trajectories using package adehabitatLT in R.[48] From these linear trajectories, we calculated net daily distance moved, which we used to estimate the movement rate (km 15 min^{-1}). We calculated Pearson product-moment correlation between ranked lead concentrations in blood and flight performance (flight height and movement rate, n = 16), and between lead concentrations in blood and progress of the moose hunting season (n = 46). The lead concentration in blood (70 ppb ww at date of tagging) of the eagle that was killed by collision with train (85 ppb ww at date of death) was not included in the visualizations and analyses since it was an outlier as it was recovered 139 days after start of moose hunting season while all other values were derived from <80 days from the start of that season. The overall results and conclusions were however not affected by including or excluding data from this eagle.

We used analysis of variance (ANOVA) on ranked lead concentrations to explore whether the measured lead concentrations in liver per category of cause of death, in the specimen bank sample of Golden Eagles found dead, differed between categories. Statistical analyses were performed in R.[49]

RESULTS

Mean observed flight height in the 16 Golden Eagles ranged from 18.4 to 74.1 m with rather narrow 95% confidence intervals (CI) for 15 of the eagles ranging from 1.4 to 11.8 m, while one eagle had a CI of 20.3 m (Table 1). Mean movement rate in the eagles ranged from 0.05 to 1.10 km 15 min^{-1} with CI of 0.02−0.19 km 15 min^{-1} (Table 1). Mean flight height and mean movement rate in the 16 Golden Eagles were nonlinearly and negatively related to the eagles' blood lead concentrations (Figure 1). Applying the Benchmark Dose Response approach, we suggest a critical (sublethal) lead concentration in blood of 25 ppb ww (wet weight) based on a 10% reduction in flight height (Figure 1). At concentrations around the inflection point of the curve (c. 43 ppb ww), flight height was reduced by 20% and in the birds with highest concentrations it was reduced by 50% (Figure 1). Lead concentrations in blood increased with progress of the hunting season (r = 0.896, n = 46, P < 0.001) and increased in two Golden Eagles that died during our study (Figure 2).

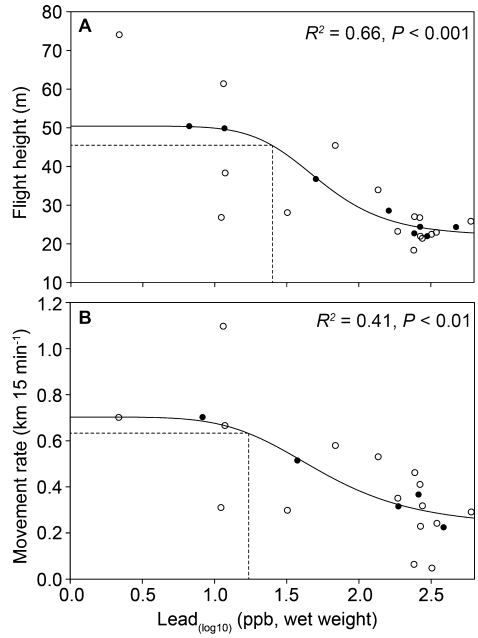


Figure 1. Relationships between movement of free ranging Golden Eagles and lead concentrations in blood. (*A*) Mean flight height, (*B*) mean movement rate of 16 adults (open circles) during 14 days after tagging. Pearson coefficients of determination are given. Continuous lines show sigmoid fit of mean movement values (filled circles) of at least two (flight height) or three (movement rate) observations for each of the mean values (see Materials and Methods). The intersection between dashed horizontal lines and *y*-axes shows when the movement variable decreased 10% below the "normal value", and the intersection between vertical dashed lines and *x*-axes shows corresponding log-lead concentration for sublethal effects (A: 1.40 ≈ 25 ppb, B: 1.23 ≈ 17 ppb lead).

As a comparison with our tagged study eagles, we also evaluated post mortem lead concentrations in liver for a larger sample of recovered dead Golden Eagles during 2003−2011 (n = 103) (Figure 3). There was a strong tendency for differing lead concentrations among the categories of mortality (ANOVA, F_7 = 2.02, P = 0.06), with highest concentrations in the categories starvation, rail and road vehicle collisions and other trauma and with a large variation in concentrations in each category (Figure 3). In total, six of the recovered dead eagles (including the eagle that collided with a wind turbine) had lead liver concentrations higher than the hitherto assumed threshold for subclinical lead concentrations (2000 ppb ww[17]) (Figure 3).

Three tagged eagles were later recovered dead during our study. Lead concentrations in liver of the three specimens at time of death were 316 (died most likely due to collision with powerline under which it was found), 346 (killed by collision with train) and 1820 (died from chronic lead poisoning and showed clear signs of starvation) ppb ww, respectively. The cause of death of these three eagles was determined by SVA. In addition, for two of the three eagles, lead concentrations in blood more than doubled from date of tagging to date of death. For the eagle that was clinically poisoned, blood lead concentration was 267 ppb ww at the time of tagging and

DOI: 10.1021/acs.est.6b06024
Environ. Sci. Technol. 2017, 51, 5729−5736

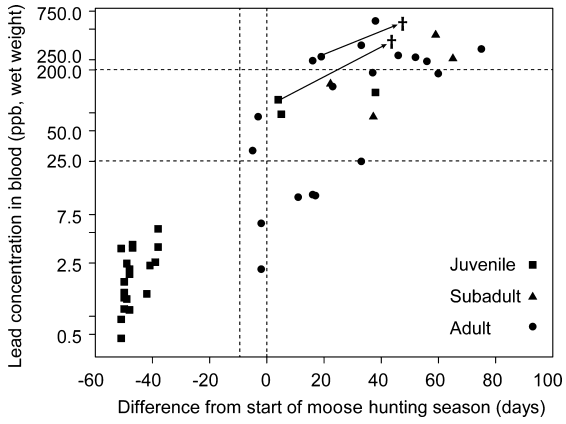


Figure 2. Lead concentration in blood of 46 free-ranging Golden Eagles (23 juveniles, four subadults, and 19 adults) as a function of progress of the moose hunting season in Sweden. Dashed vertical 0-line indicates start of the moose hunting season (first Monday in September), −10-line start of the small game hunting season; dashed horizontal lines represent previously considered boundary between "background" and sublethal lead concentrations (200 ppb lead, wet weight), and suggested new boundary (25 ppb lead, wet weight; cf. Figure 1), respectively. Arrows illustrate change in lead concentration in blood from tagging to death of two eagles (cross-marked).

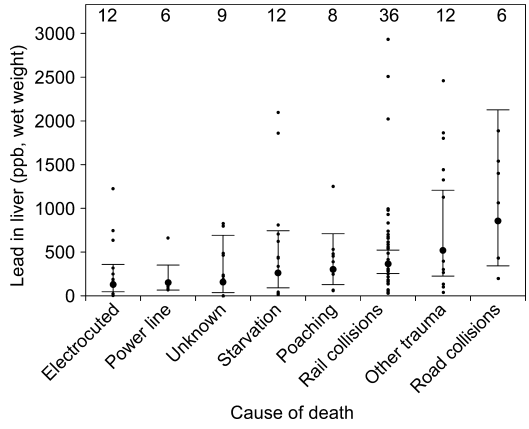


Figure 3. Post mortem lead concentrations in liver of 101 Golden Eagles recovered in 2003−2011 in Sweden. Eagles are classified by cause of death and sorted in ascending order of mean lead content in liver (geometric means (big dots) ± 95% confidence intervals; small dots = individual values).

increased in 28 days to 573 ppb ww at date of death. For the eagle that most likely died due to collision with powerline (showing physical trauma to the skull), lead concentrations increased almost 4-fold in 39 days from 101 ppb ww at date of tagging to 389 ppb ww at date of death (Figure 2).

DISCUSSION

Although lead-induced behavioral effects have been predicted previously,[6,7,18] we identified such effects in free-ranging animals for the first time. From our study on free-ranging eagles and post mortem analyses (including two of the three eagles that died during our study and that had sublethal blood lead concentrations at the date of tagging), we infer that sublethal lead concentrations impair flight performance and increase mortality risk. The here suggested critical (sublethal) lead concentration in blood of 25 ppb ww (wet weight) (Figure 1) is as little as $^{1}/_{8}$ of the generally accepted lower boundary between "background" and subclinical concentrations of 200 ppb lead ww in blood.[17] Thus, our finding is a significant advancement of the knowledge on sublethal effects of lead on eagles, and therefore also an important basis for future conservation policy. Because of our small sample size, we suggest that the estimated BMD should be treated with caution until additional studies with larger samples sizes are conducted.

Lead-based ammunition has been identified as the main anthropogenic source of lead found in the blood of wildlife.[5,17] We postulate that lead from ammunition is also the primary source for sublethal lead poisoning likely affecting Golden Eagles in Sweden. This statement is supported by (a) the observed impaired flight performance in terms of decreased flight height and movement rate with increasing concentrations of lead in the blood (Figure 1) and (b) the strong positive correlation between progress of the moose hunting season and lead concentrations in blood of tagged eagles, including the increase in blood lead concentrations in the two eagles that died during our study (Figure 2). Considering the short half-life of lead in blood (14 days),[50] the increase of lead in blood in the three Golden Eagles that died during our study likely indicates recurrent ingestion of lead-poisoned food.

Moose hunting for meat is a landscape-scale cultural activity in Fennoscandia, and annually about 150 000 to 200 000 moose are shot.[51,52] The hunting period is fixed throughout the region, and annual quotas are designated to moose management areas administered by local and national authorities. Golden Eagles exhibit a foraging response to the availability of moose gut-piles and cutoffs during the hunting season.[4] During the 2013−2014 hunting season, a total of 166 000 moose were harvested in Sweden (57%), Finland (23%), and Norway (20%). An estimated amount of 215 kg of lead from spent ammunition was deposited in gut piles, offal, and carcasses left for scavenging in one year, corresponding to more than 100 000 lethal doses for eagles.[53,54] Similar data for other game hunted with lead-based ammunition have not been reported. The annual amount of lead available for scavenging in Fennoscandia highlights lead exposure as a severe problem. This problem is even more apparent considering the extent of the use of lead-based ammunition for big game hunting worldwide.[14,55] Eagles are known to be readily attracted to carcasses.[56] This can lead to temporal reductions in their flight activity. It could be argued that a reduction in flight activities in Golden Eagles after the onset of the moose hunt might result from the access to a "new", immobile food resource (gut piles) during autumn. But this is unlikely, because gut piles are rapidly eaten and removed locally by a variety of scavengers including eagles. Golden Eagles utilizing this food source must still actively search for food on a daily, or near so, basis. Unfortunately, there are no data on the availability and spatial and temporal distribution of gut piles in our study area to test this alternative hypothesis.

We infer big game hunting as the main cause of lead poisoning and a driver of lead-induced behavioral changes and mortality in Golden Eagles. However, parallel small game hunting and wounding of, for example, mountain hare, forest grouse (*Tetrao urogallus* L., *Lyrurus tetrix* L., *Lagopus lagopus* L., and *Bonasa bonasia* L.), roe deer (*Capreolus capreolus* L.), and red fox (*Vulpes vulpes* L.), also occurring outside the big game hunting season further increases the exposure of Golden Eagles and other wildlife to lead.[23] The Golden Eagle is a long-lived species, occasionally living up to 30 years or more, but with low annual productivity.[28] Hence, lead-induced mortality or lead-reduced breeding success can be detrimental for Golden Eagle

DOI: 10.1021/acs.est.6b06024
Environ. Sci. Technol. 2017, 51, 5729−5736

populations, where individuals generally do not start reproducing until an age of >5 years.[28]

The hitherto assumed thresholds for toxic lead concentrations in blood of scavengers[5] have never been tested on free-ranging eagles. In contrast to captive birds, wild birds are expected to tolerate lower lead concentrations due to simultaneous exposure to multiple environmental stressors simultaneously.[17] In addition, long-lasting exposure of birds to low lead concentrations may be just as serious as short-term exposure to high lead concentrations.[17] Our results (Figure 1) indicate that flight performance was impaired in wild birds even at lead concentrations as low as $^{1}/_{8}$ of those currently considered as elevated.[17] This stresses the importance of including ecologically relevant end points, such as altered behavior, when determining threshold levels in wild birds and also the need to consider multiple and indirect effects of contaminants. Other recent studies on the effects of lead on physiology and body condition of waterfowl point in the same direction as our results on Golden Eagles, and also support that lead concentrations currently accepted as background levels need to be revised.[22,57] Likewise, while developing a threshold value for lead in liver, one needs to consider potential sublethal effects that might be related to increased risk of mortality. If such a revised threshold level for lead in liver were as low as the threshold level in blood that we identified (i.e., $^{1}/_{8}$ of the hitherto assumed threshold of 2000 ppb ww = 250 ppb ww lead in liver), as many as 65 of the recovered 103 dead eagles in this study would show sublethal lead concentrations (Figure 3). Although this boundary is speculative, it is clearly in line with the lead concentration in liver of the three tagged eagles that died during our study (316, 346 and 1820 ppb ww), likely implying that sublethal lead concentrations might also reduce hunting success and alertness (resulting in starvation), and not only increase collision risk. Our results urge for increased focus to also identify sublethal lead concentration thresholds in liver.

An important complementary next step for future studies will be to monitor lead concentrations in blood and flight behavior of both juveniles and adults, by repeated sampling of the same individuals before and over the course of the hunting seasons. In addition, knowledge on the spatial and temporal distribution on hunted game across the landscape would further improve our understanding of the relationship between behavior and lead.

Even though our study focused on Golden Eagles, we expect similar adverse demographic and behavioral effects of lead from wildlife hunting on other scavenging species such as corvids,[4] foxes, brown bear (*Ursus arctos* L.), wolverine (*Gulo gulo* L.), and other raptors in the boreal region.

The negative effects of lead on wildlife have been recognized for more than 150 years and from the 1930s, lead poisoning in waterfowl has been widely acknowledged.[58] Despite these early warnings, initiatives for banning lead ammunition came first in the 1970s and in the U.S. the use of lead shot for hunting of waterfowl was banned in 1991.[5,58] By 2009, 26 countries had restricted the use of lead ammunition for hunting.[59] After first banning lead shot in 2005, the Norwegian parliament recently (3 February 2015) repealed the ban of lead shot for wildlife hunting outside wetlands. Our study suggests that this type of decision is likely to have negative impacts on the Scandinavian Golden Eagle population as well as on other wildlife. We therefore strongly advocate that (a) background lead levels, in accordance with this and other studies[22,57] should be revised and (b) only lead-free ammunition should be allowed for game hunting.[14] Until lead-based ammunition is abandoned, there is also a need for legislation against leaving lead-contaminated gut piles and offal from big game hunting available for scavengers to feed on.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b06024.

Details on chemical analyses (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +46-70-2636155; fax: +46-90-7868162; e-mail: Frauke.Ecke@slu.se.

ORCID

Frauke Ecke: 0000-0003-4208-345X

Navinder J. Singh: 0000-0002-5131-0004

Author Contributions

∞F.E. and N.J.S. contributed equally to this work.

Notes

The authors declare no competing financial interest.

ÅHans Borg: Deceased in 2015

■ ACKNOWLEDGMENTS

This work was funded by the Vindval program of the Swedish Energy Agency (Dnr: 2013-001098, Dnr: 2014-005051) and administrated by the Swedish Environmental Protection Agency (SEPA). The lead analyses of liver samples were funded by SEPA (Dnr NV-10828-11) and administered by the Swedish Museum of Natural History and ACES, Stockholm University. Marcus Sundbom, ACES, provided the methods for analysis of lead in liver. The comments of four anonymous reviewers helped improving the manuscript. Tim Hipkiss improved the English.

■ REFERENCES

(1) Ogada, D. L.; Torchin, M. E.; Kinnaird, M. F.; Ezenwa, V. O. Effects of Vulture Declines on Facultative Scavengers and Potential Implications for Mammalian Disease Transmission. *Conservation Biology* **2012**, *26* (3), 453−460.

(2) Finkelstein, M. E.; Doak, D. F.; George, D.; Burnett, J.; Brandt, J.; Church, M.; Grantham, J.; Smith, D. R. Lead poisoning and the deceptive recovery of the critically endangered California condor. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (28), 11449−11454.

(3) Church, M. E.; Gwiazda, R.; Risebrough, R. W.; Sorenson, K.; Chamberlain, C. P.; Farry, S.; Heinrich, W.; Rideout, B. A.; Smith, D. R. Ammunition is the Principal Source of Lead Accumulated by California Condors Re-Introduced to the Wild. *Environ. Sci. Technol.* **2006**, *40* (19), 6143−6150.

(4) Legagneux, P.; Suffice, P.; Messier, J.-S.; Lelievre, F.; Tremblay, J. A.; Maisonneuve, C.; Saint-Louis, R.; Bêty, J. High Risk of Lead Contamination for Scavengers in an Area with High Moose Hunting Success. *PLoS One* **2014**, *9* (11), e111546.

(5) Golden, N. H.; Warner, S. E.; Coffey, M. J., A Review and Assessment of Spent Lead Ammunition and Its Exposure and Effects to Scavenging Birds in the United States. In *Reviews of Environmental Contamination and Toxicology*; de Voogt, W. P., Ed.; Springer International Publishing, 2016; Vol. *237*, pp 123−191.

(6) Helander, B.; Axelsson, J.; Borg, H.; Holm, K.; Bignert, A. Ingestion of lead from ammunition and lead concentrations in white-tailed sea eagles (*Haliaeetus albicilla*) in Sweden. *Sci. Total Environ.* **2009**, *407* (21), 5555−5563.

(7) Kelly, A.; Kelly, S. Are Mute Swans with elevated blood lead levels more likely to collide with overhead power lines? *Waterbirds* **2005**, *28* (3), 331−334.
(8) Morales, J. M.; Moorcroft, P. R.; Matthiopoulos, J.; Frair, J. L.; Kie, J. G.; Powell, R. A.; Merrill, E. H.; Haydon, D. T. Building the bridge between animal movement and population dynamics. *Philos. Trans. R. Soc., B* **2010**, *365* (1550), 2289−2301.
(9) Nathan, R.; Getz, W. M.; Revilla, E.; Holyoak, M.; Kadmon, R.; Saltz, D.; Smouse, P. E. A movement ecology paradigm for unifying organismal movement research. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (49), 19052−19059.
(10) Brodin, T.; Piovano, S.; Fick, J.; Klaminder, J.; Heynen, M.; Jonsson, M. Ecological effects of pharmaceuticals in aquatic systems—impacts through behavioural alterations. *Philos. Trans. R. Soc., B* **2014**, *369* (1656), 20130580.
(11) Lanzone, M. J.; Miller, T. A.; Turk, P.; Halverson, C.; Maisonneuve, C.; Tremblay, J. A.; Cooper, J.; O'Malley, K.; Brooks, R. P.; Katzner, T. E. Flight responses by a migratory soaring raptor to changing meteorological conditions. *Biol. Lett.* **2012**, *8* (5), 710−713.
(12) Singh, N. J.; Börger, L.; Dettki, H.; Bunnefeld, N.; Ericsson, G. From migration to nomadism: movement variability in a northern ungulate across its latitudinal range. *Ecological Applications* **2012**, *22* (7), 2007−2020.
(13) Evans, A. L.; Singh, N. J.; Friebe, A.; Arnemo, J. M.; Laske, T. G.; Fröbert, O.; Swenson, J. E.; Blanc, S. Drivers of hibernation in the brown bear. *Front. Zool.* **2016**, *13* (1), 7.
(14) Bedrosian, B.; Craighead, D.; Crandall, R. Lead Exposure in Bald Eagles from Big Game Hunting, the Continental Implications and Successful Mitigation Efforts. *PLoS One* **2012**, *7* (12), e51978.
(15) Holmstad, P. R. Do bag records of willow ptarmigan underestimate hunting mortality? (In Norwegian with English summary). *Fauna (Oslo)* **1998**, *51* (3), 94−96.
(16) Larsen, S.; Nybakk, D. *Riflejakt som jaktform på hare (Lepus timidus) og omfanget av skadeskytning med haglegevær på hare (Lepus timidus) (in Norwegian with English summary)*; Høgskolen i Hedmark, 2011.
(17) Franson, J. C.; Pain, D. J., Lead in birds. In *Environmental Contaminents in Biota: Interpreting Tissue Concentrations*; Beyer, W. N., Ed. CRC Press: Boca Raton, FL, 2011; pp 563−593.
(18) Burger, J.; Gochfeld, M. Effects of lead on birds (Laridae): A review of laboratory and field studies. *J. Toxicol. Environ. Health, Part B* **2000**, *3* (2), 59−78.
(19) Cruz-Martinez, L.; Redig, P.; Deen, J. Lead from spent ammunition: a source of exposure and poisoning in bald eagles. *Human-Wildlife Interactions* **2012**, *6*, 94−104.
(20) Hoffman, D. J.; Pattee, O. H.; Wiemeyer, S. N.; Mulhern, B. Effects of lead shot ingestion on d-aminolevulinic acid dehydratase activity, hemoglobin concentration and serum chemistry in bald eagles. *J. Wildl. Dis.* **1981**, *17* (3), 423−431.
(21) Henny, C. J.; Blus, L. J.; Hoffman, D. J.; Grove, R. A.; Hatfield, J. S. Lead accumulation and osprey production near a mining site on the coeur d'Alene River, Idaho. *Arch. Environ. Contam. Toxicol.* **1991**, *21* (3), 415−424.
(22) Martinez-Haro, M.; Green, A. J.; Mateo, R. Effects of lead exposure on oxidative stress biomarkers and plasma biochemistry in waterbirds in the field. *Environ. Res.* **2011**, *111* (4), 530−538.
(23) Kramer, J. L.; Redig, P. T. Sixteen years of lead poisoning in eagles, 1980−95: an epizootilogic view. *J. Raptor Res.* **1997**, *31* (4), 327.
(24) Neumann, K., Bald eagle lead poisoning in winter. In *Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans*; Watson, R. T.; Fuller, M.; Pokras, M.; Hunt, W. G., Eds. The Peregrine Fund: Boise, ID, 2009.
(25) Cagnacci, F.; Boitani, L.; Powell, R. A.; Boyce, M. S. Animal ecology meets GPS-based radiotelemetry: a perfect storm of opportunities and challenges. *Philos. Trans. R. Soc., B* **2010**, *365* (1550), 2157−2162.
(26) Kays, R.; Crofoot, M. C.; Jetz, W.; Wikelski, M. Terrestrial animal tracking as an eye on life and planet. *Science* **2015**, *348* (6240), aaa2478.
(27) Hellström, G.; Klaminder, J.; Finn, F.; Persson, L.; Alanärä, A.; Jonsson, M.; Fick, J.; Brodin, T., GABAergic anxiolytic drug in water increases migration behaviour in salmon. *Nat. Commun.* **2016**, *7*.1346010.1038/ncomms13460
(28) Watson, J. *The Golden Eagle*, 2nd ed.; T & A D Poyser: London, 2010; p 448.
(29) European Union, Directive 2009/147/EC of the European Parliament and of the Council of 30 November 2009 on the conservation of wild birds. 2009.
(30) Tjernberg, M. *Artfaktablad: Aquila chrysaetos − Kungsörn*, ArtDatabanken; SLU, Uppsala, 2010.
(31) Moss, E. H. R.; Hipkiss, T.; Ecke, F.; Dettki, H.; Sandström, P.; Bloom, P. H.; Kidd, J. W.; Thomas, S. E.; Hörnfeldt, B. Home-range size and examples of post-nesting movements for adult golden eagles (*Aquila chrysaetos*) in boreal Sweden. *Journal Of Raptor Research* **2014**, *48* (2), 93−105.
(32) Tjernberg, M. Diet of the golden eagle *Aquila chrysaetos* during the breeding-season in sweden. *Holarctic Ecology* **1981**, *4* (1), 12−19.
(33) Hipkiss, T.; Ecke, F.; Dettki, H.; Moss, E.; Sandgren, C.; Hörnfeldt, B. *Betydelsen av kungsisörnars hemområden, biotopval och rörelser för vindkraftsetablering [In Swedish with English summary]*, Rapport 6589; Naturvårdsverket, 2013.
(34) Singh, N. J.; Hipkiss, T.; Ecke, F.; Hörnfeldt, B. *Betydelsen av kungsörnars hemområden, biotopval och rörelser för vindkraftsetablering. Del 2./Movement patterns of golden eagles and importance for wind power development in Sweden. Part 2*, Report 6734; Naturvårdsverket: 2017.
(35) Tjernberg, M.; Landgren, T. Kungsörn − javisst, men vilken ålder? (In Swedish). *Fåglar i Uppland* **2010**, No. 3, 5−15.
(36) Sandgren, C.; Hipkiss, T.; Dettki, H.; Ecke, F.; Hörnfeldt, B. Habitat use and ranging behaviour of juvenile Golden Eagles *Aquila chrysaetos* within natal home ranges in boreal Sweden. *Bird Study* **2014**, *61* (1), 9−16.
(37) Bloom, P. H.; Kidd, J. W.; Thomas, S. E.; Hipkiss, T.; Hörnfeldt, B.; Kuehn, M. J. Trapping success using carrion with bow nets to capture adult Golden Eagles in Sweden. *Journal of Raptor Research* **2015**, *49*, 92−97.
(38) Crump, K. S. A new method for determining allowable daily intakes. *Fundam. Appl. Toxicol.* **1984**, *4* (5), 854−871.
(39) Allen, B. C.; Kavlock, R. J.; Kimmel, C. A.; Faustman, E. M. Dose—Response Assessment for Developmental Toxicity: II. Comparison of Generic Benchmark Dose Estimates with No Observed Adverse Effect Levels. *Toxicol. Sci.* **1994**, *23* (4), 487−495.
(40) Fowles, J. R.; Alexeeff, G. V.; Dodge, D. The Use of Benchmark Dose Methodology with Acute Inhalation Lethality Data. *Regul. Toxicol. Pharmacol.* **1999**, *29* (3), 262−278.
(41) European Food Safety Authority (EFSA). Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. *EFSA J.* **2009**, *1150*, 1−72.
(42) Davis, J. A.; Gift, J. S.; Zhao, Q. J. Introduction to benchmark dose methods and U.S. EPA's benchmark dose software (BMDS) version 2.1.1. *Toxicol. Appl. Pharmacol.* **2011**, *254* (2), 181−191.
(43) US Environmental Protection Agency. *Benchmark Dose Technical Guidance Document*, Epa/100/r-12/001; EPA, 2012.
(44) Dettki, H.; Ericsson, G.; Giles, T.; Norrsken-Ericsson, M. In *Wireless Remote Animal Monitoring (WRAM) - A new international database e-infrastructure for telemetry sensor data from fish and wildlife*, Proceedings Etc. 2012: Convention for Telemetry, Test Instrumentation and Telecontrol; The European Society of Telemetry, Books on Demand:, 2013; pp 247−256.
(45) Miller, T. A.; Brooks, R. P.; Lanzone, M. J.; Brandes, D.; Cooper, J.; Tremblay, J. A.; Wilhelm, J.; Duerr, A.; Katzner, T. E. Limitations and mechanisms influencing the migratory performance of soaring birds. *Ibis* **2016**, *158* (1), 116−134.

DOI: 10.1021/acs.est.6b06024
Environ. Sci. Technol. 2017, 51, 5729−5736

(46) Reid, T.; Krüger, S.; Whitfield, D. P.; Amar, A. Using spatial analyses of bearded vulture movements in southern Africa to inform wind turbine placement. *J. Appl. Ecol.* **2015**, *52* (4), 881−892.
(47) Miller, T. Movement ecology of Golden Eagles (*Aquila chrysaetos*) in eastern North America. Penn State University, 2012.
(48) Calenge, C. *Analysis of animal movements in R: the adehabitatLT Package*, 2015.
(49) R Development Core Team. *R: A Language and Environment for Statistical Computing*. v. 3.2.4; Foundation for Statistical Computing: Vienna, Austria, 2014.
(50) Fry, M.; Sorensen, K.; Granthham, J.; Burnett, J.; Brandt, J.; Koenig, M., Lead intoxication kinetics in condors from California. In *Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans*; Watson, R. T.; Fuller, M.; Pokras, M.; Huntm, W. G., Eds.; The Peregrine Fund: Boise, ID, 2009.
(51) Lavsund, S.; Nygren, T.; Solberg, E. J. Status of moose populations and challenges to moose management in Fennoscandia. *Alces* **2003**, *39*, 109−130.
(52) Singh, N. J.; Danell, K.; Edenius, L.; Ericsson, G. Tackling the motivation to monitor: success and sustainability of a participatory monitoring programme. *Ecology and Society* **2014**, *19* (4), 7.
(53) Pattee, O. H.; Wiemeyer, S. N.; Mulhern, B. M.; Sileo, L.; Carpenter, J. W. Experimental lead-shot poisoning in Bald Eagles. *J. Wildl. Manage.* **1981**, *45*, 806−810.
(54) Stokke, S.; Brainerd, S.; Arnemo, J. Metal deposition of copper and lead in moose harvested in Fennoscandia. *Wildlife Society Bulletin* **2017**, *41* (1), 98−106.
(55) Lambertucci, S. A.; Donázar, J. A.; Hiraldo, F. Poisoning People and Wildlife with Lead Ammunition: Time to Stop. *Environ. Sci. Technol.* **2010**, *44* (20), 7759−7760.
(56) Cochrane, J. F.; Lonsdorf, E.; Allison, T. D.; Sanders-Reed, C. A. Modeling with uncertain science: estimating mitigation credits from abating lead poisoning in Golden Eagles. *Ecological Applications* **2015**, *25* (6), 1518−1533.
(57) Newth, J. L.; Rees, E. C.; Cromie, R. L.; McDonald, R. A.; Bearhop, S.; Pain, D. J.; Norton, G. J.; Deacon, C.; Hilton, G. M. Widespread exposure to lead affects the body condition of free-living whooper swans *Cygnus cygnus* wintering in Britain. *Environ. Pollut.* **2016**, *209*, 60−67.
(58) Friend, M.; Franson, J. C.; Anderson, W. L., Biological and societal dimensions of lead poisoning in birds in the USA. In *Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans*; Watson, R. T.; Fuller, M.; Pokras, M.; Hunt, W. G., Eds.; The Peregrine Fund: Boise, ID, 2009.
(59) Avery, D.; Watson, R. T., Regulation of lead-based ammunition around the world. In *Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans*; Watson, R. T.; Fuller, M.; Pokras, M.; Hunt, W. G., Eds.; The Peregrine Fund: Boise, ID, 2009.

DOI: 10.1021/acs.est.6b06024
Environ. Sci. Technol. 2017, 51, 5729−5736