ABSTRACT

Objectives. Although banned nationwide for waterfowl hunting, lead shot is still used for hunting in regions of Alaska. Consumption of birds hunted with lead shot may be a route of human lead exposure in susceptible populations. The Centers for Disease Control and Prevention (CDC) and Alaskan health officials conducted a cross-sectional exposure assessment and used isotope ratios (IR) to test that assumption.

Study Design. Cross-sectional exposure assessment study.

Methods. We compared isotopic profiles of blood lead in Alaska Native women from Bethel (n=10) and Barrow (n=10) to lead shot samples purchased from the respective regions. To evaluate the source of lead for the buckshot, we evaluated IR profiles for lead mineral and ore from a smelter in Torreon, Mexico, a suspected source of origin for the lead.

Results. The lead IRs for the blood lead differed significantly from the lead shot IRs (p< 0.001); thus, lead shot is unlikely to be the sole source of lead exposure of public health significance in participants of this study. Overlap in IRs for the lead shot and blood lead existed for 6 (30%) of the women from Bethel and Barrow; however, no correlation was noted between lead levels and the IRs for the blood lead. IR profiles for lead mineral and ore from Mexico were substantially different from the IRs of lead shot from Alaska, confirming that buckshot in this study is unlikely to originate from the Mexican smelter.

Conclusions. Lead shot from the manufacturer in this study does not appear to be the sole source of lead exposure in most participants; nonetheless, lead shot could yet be a potential source of exposure in some populations, possibly those whose diet consists of game hunted with lead shot.

Keywords: lead shot, lead isotope, lead, human, birds, seabirds
INTRODUCTION

Recent evidence suggests that consumption of birds hunted with lead shot may be a route of human lead exposure in susceptible populations (1–5). Studies have demonstrated high concentrations of lead in waterfowl (e.g., swan, geese, ducks) that ingest lead shotgun pellets deposited on the bottoms of lakes and in fields (6–8). In 1991, the U.S. Fish and Wildlife Services issued a nationwide ban on use of lead shot in waterfowl hunting and approved use of non-lead ammunition (e.g., steel and tungsten) (9). However, many hunters are reluctant to use non-lead ammunition because of its increased cost and physical characteristics that require hunters to alter their shooting methods. Because lead shot is legal for use in dry areas for other game and therefore still commercially available, use of lead shot in waterfowl hunting is pervasive in certain regions, such as western Alaska (10).

Recent studies report elevated lead concentrations in eiders, a commonly hunted waterfowl, from the Yukon-Kusokokwim Delta region of western Alaska (11,12). The Alaska Native communities living around this region (e.g., Bethel) rely largely on a subsistence diet that includes waterfowl killed with lead shot. Preliminary analysis of samples collected as part of an ongoing research project of pregnant Alaska Native women suggests that lead concentrations in Bethel are two times higher than in northern Alaska (e.g., Barrow), an area where steel shot is the predominant form of ammunition used (personal communication, Alaska health officials). Alaska health officials also suggest that the lead shot used in Bethel may originate from a lead smelter in Mexico (10).

The 4 natural stable lead isotopes are $^{204}$Pb, $^{206}$Pb, $^{207}$Pb and $^{208}$Pb (13). The relative abundances of these isotopes vary between different geologic sources of lead. These isotopic differences endure when the lead is mined and incorporated into industrial materials such as lead shot. The comparative abundance of these isotopes in any given lead source provides a “fingerprint” of the lead from that source (14,15). Because the isotope ratios (IR) remain constant, it is possible to measure differences in lead IRs from different sources. This information can be used to evaluate potential sources of exposure in human populations. If the isotope composition of lead in various sources of lead exposure is different, the difference will be reflected in the isotope composition of the blood lead IR, therefore providing an accurate method to link lead found in blood to a potential source (14,15).

The main objective of the current study was to identify whether lead shot used for hunting is a source of lead exposure among a defined population of Alaska Natives. We conducted a cross-sectional exposure assessment and used IR methodology to compare the isotopic profiles of: (1) blood lead in Alaska Native women from Bethel and Barrow; (2) lead shot samples from Bethel and Barrow; and (3) lead mineral and ore from a large smelter in Torreon, Mexico.

MATERIAL AND METHODS

We conducted the current project in 2 stages between 2003 and 2005. During the first stage, we evaluated the IR of the blood lead samples and part of the environmental samples (raw ore and lead samples from Torreon). During
the second stage, we evaluated the IR of the buckshot samples from Alaska and additional environmental lead samples from the smelter in Torreon. We then compared the IR of the different sample types to determine whether lead exposure in the study participants is from lead shot used in Alaska and whether the smelter in Torreon is the source of the lead found in the lead shot in this study. Although there was no a priori reason to suspect the Torreon smelter as a source of lead for the buckshot, previous research conducted by Alaskan health officials suggested that lead in eiders from western Alaska was similar to buckshot that originated from factories in Mexico (10). Environmental samples from Torreon were available for analysis as part of another ongoing project in that region. The Human Subjects Research Board at CDC and in Alaska approved this project.

**IR analysis**

**Instrumentation**

The IR measurements were performed on a PerkinElmer ELAN 6000 using cross-flow nebulization inductively coupled plasma mass spectrometry (ICP-MS) with the parameters provided in Table I.

**Calibration and control materials**

All observed ratios were corrected using the average of the observed isotope ratios for standard reference material (SRM) 981 natural lead (isotopic) from the National Institute of Standards and Technology (NIST). The correction was performed to eliminate mass discrimination effects (differences in sensitivity that the ELAN may exhibit between the isotopes being measured) and to normalize data collected in 2003 to the current experimental results. The SRM was prepared in triplicate in the same way that

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**Table I.** Instrument parameters for isotope ratio analysis: PerkinElmer ELAN 6000.

| Instrument and method parameters                  | Specifics                                      |
|--------------------------------------------------|------------------------------------------------|
| RF power                                         | 1.45 kW                                        |
| Nebulizer flow                                   | 0.93 LPM (pneumatic nebulizer)                 |
| Sample uptake time                               | 40 sec (1.4 mL/min, 24 rpm)                    |
| Read delay time                                  | 20 sec (1.4 mL/min, 24 rpm)                    |
| Analysis time                                    | 54 sec (1.4 mL/min, 24 rpm)                    |
| Rinse time                                       | 300 sec (1.4 mL/min, 24 rpm)                   |
| Rinse solution                                   | 5% v/v nitric acid and 5% v/v hydrochloric acid|
| Sweeps/Reading                                   | 100                                            |
| Readings/Replicate                               | 1                                              |
| Number of replicates                             | 6                                              |
| Scan mode                                        | Peak hopping                                   |
| Dwell time                                       | 15.0 ms                                        |
| Integration time                                 | 15.00 ms                                       |
| Detector mode                                    | Pulse                                          |
| Elemental/Isobaric interference correction equations | Isotope 204 was corrected for possible mercury (Hg) interference using the equation: –0.230074 x Hg 202. The correction was suggested and written by ELAN version 3.0 software. |
study samples were prepared (starting with the lead wire SRM), and was analysed at the beginning and the end of each analysis day. Corrected $^{207}\text{Pb}/^{206}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$ ratios were calculated and an average of all SRM analyses ($n=12$ over 4 days) was used in the equations for calculation of the correction factors. Correction factors were applied to the observed isotope ratios using Microsoft Excel after data acquisition.

Sample preparation

Acid digestions of each sample were prepared in triplicate. A small portion of each sample was placed into 15mL or 50mL polypropylene centrifuge tube along with approximately 10mL of 50% v/v double-distilled nitric acid. Samples were leached in the acidic solution at least overnight before dilutions were prepared for analysis. Each sample leachate was diluted two to three times in 15mL or 50mL polypropylene tubes using 5% v/v double-distilled nitric acid before being analysed for lead isotope ratios on the ICP-MS.

ICP-MS analysis

After daily optimization and checking for background lead levels in the ICP-MS, a new 5% v/v nitric acid blank was analysed each day of analysis. NIST SRM 981 was analysed at the beginning and the end of each day of analysis as a quality control check. Dilutions for each sample leachate were customized to provide approximately 500,000–1,000,000 total counts per second (cps) for all lead isotopes during analysis. Random study samples were analysed on more than 1 day as a check for reproducibility of results.

Blood lead samples

The Alaska Native participants in this study were pregnant women who were enrolled as part of a broader multi-year program in Bethel and Barrow. The primary objective of the multi-year program is to monitor selected persistent organic pollutants and heavy metals in umbilical cord blood and maternal blood of an Alaska Native population that predominantly relies on a subsistence diet. Blood is collected from pregnant Alaska Native women in Bethel and Barrow during their first prenatal visit. A convenience sample of participants from Bethel ($n=10$) and Barrow ($n=10$) was selected for this study of lead exposure on the basis of volume of blood available in the stored samples from the multi-year program. Similarly, blood samples from Torreon ($n=10$) were collected from children living less than 1.5 miles from the lead smelters (described below) as part of another ongoing project in that region.

Whole blood samples were collected for all study participants in evacuated tubes that were pre-screened for lead contamination. The syringes, needles, butterflies, alcohol wipes and gauze pads were also pre-screened for lead contamination before use. All blood samples were kept at 4°C during storage and transport.

Lead shot samples

Twenty-eight buckshot samples were analysed in this study. They were convenience samples belonging to a single manufacturer and were obtained from the local stores by Alaskan officials. We are not aware of the sources for the lead used to manufacture the buckshot.
Lead shots and human exposure

Ore and processed lead samples
Two ore samples and 1 processed lead sample were collected from a smelter in Torreon, Mexico. These samples were provided to one of the authors (Jones) by the medical officer of the lead smelter during a public health investigation in Torreon in 2003.

Data analysis
We used descriptive statistics to characterize blood lead concentrations and lead IR variability within the media. We used the Wilcoxon rank sum test to compare lead IR within blood sample groups and across media. Although we calculated, reviewed and presented IRs for $^{204}\text{Pb}/^{206}\text{Pb}$ ratio on all samples, we did not conduct statistical data analysis on $^{204}\text{Pb}/^{206}\text{Pb}$ ratios because of multiple outliers in the Barrow blood samples. Samples from Barrow had low blood lead concentrations (mean = 1.39µg/dL) leading to increased variability in the repeated measurements of the $^{204}\text{Pb}/^{206}\text{Pb}$ ratios (relative standard deviations [RSD] range = 0.1% to 14.9%). Data analyses were conducted using SAS statistical software v. 9.0 (SAS Institute, Cary, NC) and Microsoft Excel.

RESULTS
The mean blood lead concentration in Bethel samples was 4.72µg/dL (95% CI=3.76–5.68) and 1.39µg/dL (95% CI=1.08–1.70) in Barrow samples. Table II lists the mean lead IRs for the blood, lead shot, ore and processed lead samples. The lead IRs for the lead shot were significantly different from those observed for the blood lead samples in Bethel (p<0.001) and Barrow (p<0.001) (Table III). The analysis and Figure 1 suggest that the source of blood lead exposure in these participants is unlikely to be lead shot obtained for testing in this study.

The lead IRs for blood samples from Torreon are significantly different from those of blood samples from Bethel (p=0.023) and Barrow (p<0.001). This implies that the women sampled

| Table II. Mean lead concentrations and isotope results for blood, buckshot, ore and processed lead. |
|-----------------------------------------------|
| Sample type                  | Isotope Ratio Mean (95% CL) |
|                              | $^{204}\text{Pb}/^{206}\text{Pb}$ | $^{207}\text{Pb}/^{206}\text{Pb}$ | $^{208}\text{Pb}/^{206}\text{Pb}$ |
| Blood lead                   |                             |                             |                             |
| Pregnant women – Bethel, AK  | 0.0526 (0.0517, 0.0535)      | 0.8340 (0.8207, 0.8472)      | 2.0445 (2.0219, 2.0670)     |
| (n=10, mean*=4.72µg/dL)      |                             |                             |                             |
| Pregnant women – Barrow, AK  | 0.0516 (0.0500, 0.0531)      | 0.8254 (0.8198, 0.8311)      | 2.0387 (2.0247, 2.0528)     |
| (n=10, mean†=1.39µg/dL)      |                             |                             |                             |
| Children – Torreon, Mexico  | 0.0536 (0.0533, 0.0538)      | 0.8398 (0.8381, 0.8416)      | 2.0725 (2.0680, 2.0770)     |
| (n=10)                       |                             |                             |                             |
| Environmental                |                             |                             |                             |
| Lead buckshot (AK)           | 0.0518 (0.0515, 0.0521)      | 0.8132 (0.8090, 0.8173)      | 2.0009 (1.9934, 2.0084)     |
| (n=28)                       |                             |                             |                             |
| Ore, sample A ‡ (n=1)        | 0.0539 (0.0515, 0.0521)      | 0.8399                      | 2.0686                      |
| Ore, sample B ‡ (n=1)        | 0.0538                      | 0.8399                      | 2.0700                      |
| Processed lead sample‡ (n=1) | 0.0535                      | 0.8353                      | 2.0727                      |

* Range=3.40–7.90.  
† Range=1.00–2.30.  
‡ From smelter in Torreon, Mexico.  
Pb denotes lead; CL denotes confidence limits; AK denotes Alaska.
Lead shots and human exposure

in Alaska were exposed to lead that originated from a different source than the lead to which persons in Torreon were exposed (Table II).

We compared lead IR for blood, lead shot, ore and processed lead samples in this study (Fig. 1). Because the sample size for the smelter lead samples was too small (n=3) for statistical comparison, the plot was used for comparison of these samples with lead IRs of blood and lead shot samples. The plot indicates that lead IRs for the smelter lead samples and the lead shot samples are different, thus making it unlikely that the smelter ore is the sole source of lead used to manufacture the buckshot. Torreon blood samples have a similar IR as the ore and processed lead samples that were obtained from smelters in the same region. For most people in Bethel and Barrow (70%), the lead IR differs from the lead-shot and the environmental and blood samples from Torreon (Fig. 1).

Table III. Comparisons of \(^{207}\text{Pb}/^{206}\text{Pb}\) ratios among all sample types: blood and lead shot samples.

| Sample type       | Barrow—blood lead† (pregnant women) | Torreon—blood lead‡   | Lead buckshot |
|-------------------|-------------------------------------|-----------------------|---------------|
| Bethel—blood lead† | p=0.446                             | p=0.023               | p<0.001       |
| Barrow—blood lead‡ | p<0.001                             | p<0.001               |               |

* Wilcoxon rank sum test was used for analysis.
†Blood from pregnant women.
‡Blood from children.
Pb denotes lead; Bethel and Barrow are in Alaska; Torreon is in Mexico; blank cell denotes redundant value.

Figure 1. Comparison of lead isotopic ratios for blood, environmental and lead-shot samples.*

* Two outliers (one lead shot and one blood lead) samples are not shown on this graph to allow for enhanced visualization of the remaining isotopic ratios. Processed lead, lead ore sample A and lead ore sample B refer to environmental samples from a smelter in Torreon, Mexico.
DISCUSSION

There are two predominant findings of this study. First, the lead IR comparisons suggest that the lead-shot samples analysed in this study are unlikely to have been a significant source of lead exposure in the participants of this project. Second, the lead from the smelter in Torreon, Mexico, is unlikely to be used for manufacturing the buckshot samples in this study.

Figure 1 illustrates that although there are overlapping points in the lead IRs of the various samples, most of the data points aggregate according to their respective group. The statistical analysis, where we compared the average IRs of the lead shot with the average IRs of the blood specimens, confirms that these IRs are indeed different. The difference in IRs suggests that lead shot is unlikely to contribute significantly to the source of the lead in most of the blood lead specimens from Bethel and Barrow. However, the overlap in IRs for the lead shot and 6 (30%) of the women from Bethel and Barrow suggests that lead shot could yet be a potential source of exposure in some populations, possibly those whose diet consists of game hunted with lead shot.

Because of the small sample size of smelter lead samples (n=3), we did not conduct statistical comparisons of these samples with the blood and lead shot samples. However, the figures illustrate that blood specimens from Torreon have similar IRs as the smelter lead and that the lead shot samples do not. This indicates that lead used for manufacturing of buckshot in this study is unlikely to be from the Mexican smelter.

Data are sparse on sources of lead exposure in Alaska. Lead paint is rare in Alaska since 93% of the homes were built after 1950. Studies from other Arctic regions suggest diet as a possibility, although occasional reports exist of situational lead exposure at indoor firing ranges and through medicinal use (2–5,16,17). Lead IR analysis is often used for identifying sources of lead exposure (13–15). The ideal conditions where lead IR analysis would be beneficial are high lead concentrations in biological specimens, limited sources of lead exposure, and availability of corresponding environmental samples that are suspected sources of exposure.

There are several limitations of this project. The small sample size and the low lead concentrations resulted in increased variance in the lead IR results. Nonetheless, the differences in the IRs between the blood and the lead shot samples were statistically significant (Table III) and apparently different on the plot (Fig. 1). Another major limitation is that the blood samples were from a pregnant population and not a general Alaska Native population, specifically ones that are known consumers of game that might be hunted using lead shot. The small sample size and its homogenous nature limit the application of the results to the general Alaskan population.

If 2 or more sources of lead exist in blood, the resulting lead IR would be mixed and would reflect the combined IRs of the mixed sources. Although the IRs of the lead shot and the blood from women in Bethel and Barrow appear to be different for most of the samples (Fig. 1), we cannot exclude the possibility that buckshot partially contributed to the blood lead concentrations, particularly in the 6 (30%) women whose IRs overlap those of the lead shot.
Conclusions

Lead shot from the manufacturer in this study does not appear to be the sole source of lead exposure in most participants of this study. However, the possibility exists that lead shot could contribute to a portion of the lead exposure particularly among persons relying on game hunted with lead shot. Studies with a larger sample specifically among high risk groups, with a comparison population, are necessary to further assess whether lead shot is a source of blood lead concentrations in Alaska Natives who have elevated blood lead concentrations and who rely on game hunted with lead shot.

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