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High-Precision (MC-ICPMS) Isotope Ratio Analysis Reveals Contrasting Sources of Elevated Blood Lead Levels of an Adult with Retained Bullet Fragments, and of His Child, in Milwaukee, Wisconsin

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Abstract Exposure to the neurotoxic element lead (Pb) continues to be a major human health concern, particularly for children in US urban settings, and the need for robust tools for assessment of exposure sources has never been greater. The latest generation of multicollector inductively coupled plasma mass spectrometry (MC-ICPMS) instrumentation offers the capability of using Pb isotopic signatures as a tool for environmental source tracking in public health. We present a case where MC-ICPMS was applied to isotopically resolve Pb sources in human clinical samples. An adult male and his child residing in Milwaukee, Wisconsin, presented to care in August 2015 with elevated blood lead levels (BLLs) (>200 μg/dL for the adult and 10 μg/dL for the child). The adult subject is a gunshot victim who had multiple bullet fragments embedded in soft tissue of his thigh for approximately 10 years. This study compared the high-precision isotopic fingerprints (<1 ‰ 2σ external precision) of Pb in the adult's and child's whole blood (WB) to the following possible Pb sources: a surgically extracted bullet fragment, household paint samples and tap water, and a Pb water-distribution pipe removed from servicing a house in the same neighborhood. Pb in the bullet and adult WB were nearly isotopically indistinguishable (matching within 0.05–0.56 ‰), indicating that bullet fragments embedded in soft tissue could be the cause of both acute and chronic elevated blood Pb levels. Among other sources investigated, no single source dominated the child's exposure profile as reflected in the elevated BLL.

Keywords Pb isotopes · MC-ICPMS · Blood lead · Bullet · Public health · Pb sources

Introduction

Lead (Pb) stable isotopic fingerprinting in geochemical source tracking is a relatively mature field of study with reports describing geochemical applications throughout multiple decades. Measuring Pb isotope variations has been used to examine and constrain everything from large-scale geochemical and environmental processes [1–4] to source-tracking household and biological hazards that are geologically or anthropogenically sourced (i.e., water, aerosols, leaded gasoline, or Pb for bullets, paint, and ceramic glazes) [5–12].

Pb stable isotope work on biological and clinical samples, although not absent from the literature, is rare [7, 13–15], especially the application of ultrahigh-precision isotope ratio analysis made possible by the new generation of multicollector inductively coupled plasma mass spectrometry (MC-ICPMS) instrumentation. The majority of previous work in this field did not use a multicollector instrument and lacks the isotope ratio precision to do much beyond making broad comparisons among different Pb reservoirs and Pb isotopes in blood [7, 14, 15]. Furthermore, validity (especially the potential for isotopic fractionation biases) of Pb isotope data on

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unspiked samples obtained by MC-ICPMS and associated sample processing has been primarily demonstrated on geologic samples [16, 17]. Thus, implementation of MC-ICPMS to clinical samples (as in this study) was not routine. To realize and document the potential of the methods, further method validation was needed, especially because extensive sample cleanup is required to take advantage of the ultrahigh precision possible with the MC mass spectrometer, and rarely has whole blood (WB) (a difficult sample matrix) been processed for MC-ICPMS analysis.

Personnel from the Bureau of Environmental and Occupational Health of the Wisconsin Division of Public Health, Department of Health Services (DHS), presented a unique case of extremely elevated blood lead level (BLL) to the trace element research staff at the Wisconsin State Laboratory of Hygiene (WSLH) for consultation and analytical assistance; a man aged 30 years was evaluated in August 2015 in an emergency department with a 1-month history of severe abdominal pain, jaundice, constipation, lower extremity weakness, and weight loss. Multiple retained bullet fragments were identified in the soft tissues of the upper left thigh from a gunshot wound that had been sustained approximately 10 years previously. One month before symptom onset, the patient had been involved in a car accident; he reported that he had previously been able to feel one of the larger bullet fragments under the skin of his thigh, but could no longer feel it after the accident [18]. The patient had an initial BLL of >200 μg/dL, recommended reference level is 5 μg/dL [19], and to lower the Pb burden the patient started a chelation regimen followed by surgical excision of the largest bullet fragment.

DHS was interested in ascertaining whether the bullet was the likely source of the elevated BLL, a case of special interest because bullet retention in soft tissues is not usually considered a risk factor for elevated blood Pb levels [20, 21]. Weiss et al. [18] hypothesized that the car accident might have disrupted one or more of the embedded fragments (and ruptured associated cysts containing high levels of Pb) and contributed to Pb exposure. In addition to applying Pb isotope methods to address this question, we analyzed the blood of the patient's child (2 years old as of summer 2016), who resided in the same household; the child had a BLL of 10 μg/dL. We investigated other possible Pb sources that the adult and child might have been exposed to in hopes of identifying the Pb source(s) and included two paint samples, tap water, and a section of Pb water distribution pipe, a service lateral from the same neighborhood. Interviews with household members did not identify additional, substantial occupational or other Pb exposures [18].

Sample Collection and Custody

WB samples (in lavender top, EDTA tubes) from the adult male (n = 4) and child (n = 2) were delivered to WSLH. A substantially deformed bullet fragment from the adult male was surgically removed and rinsed free of tissue and blood. The bullet, along with residual saline, was placed into a polypropylene sample container and delivered to WSLH. Two paint samples (scrapings of peeling paint) from the home of the adult subject (a four-unit apartment building) were collected from a window sill (paint A) and a door (paint B). The scrapings consisted of multiple layers of paint, including both flat and high-gloss finishes. Scrapings were placed into polypropylene sample containers and delivered to WSLH. A first-flush tap water sample from the home was collected by using Milwaukee Public Health and Environmental Protection Agency (EPA) guidelines. The sample was collected after 12 h of stagnation, and no additional water treatment devices on the drinking water supply line were noted. A section of lead water pipe (circa 1916), similar to that of the service line to the subject's home and from the same neighborhood, was also obtained. The Milwaukee Water Works confirmed that although the apartment building was built in 1954, it does have Pb service laterals, and the 1916 water pipe was the only available proxy for those service laterals at the time.

Analytical Methods

Sample Preparation

All sample handling and processing (with the exception of the total Pb analysis in WB, paint, and bullet) was performed in purpose-built, dedicated trace element clean laboratory (TECL) at WSLH. Established protocols that substantially minimize the potential for isotope fractionation and contamination were executed by WSLH staff with substantial experience in modern trace element methods [1, 10]. Only high-purity, Optima™-grade (Thermo Fisher Scientific, Incorporated, Waltham, MA) acids and 18.2 MΩ cm water were used for the clean laboratory sample processing. Use of certified reference materials (CRMs), both spiked and unspiked, allowed us to determine which digestion and sample cleanup methods were best for each sample matrix. Sufficient mass of sample was digested such that >75 ng Pb was present in the final digest, which would allow for multiple analyses for Pb isotopes and other elements, if necessary.

Whole Blood

Aliquots of WB samples were analyzed for total Pb in the clinical metal section of WSLH by using the method of Parsons and Slavin [22]. Additional WB samples were aliquoted in the clean laboratory (0.25–0.75 mL of WB, depending on sample) and oven-digested at 85 °C in a closed vessel with 5 mL of HNO_3 and 1 mL of H_2O_2 for at least 16 h.

Pb Source Materials

1. *Bullet*. The bullet was rinsed in ultrapure water, then multiple flakes were shaved from the bullet with a ceramic blade. Three separate samples of 20–40 mg each were brought completely into solution by using 30 % (*v*/*v*) HNO_3 in a closed vessel at room temperature during the course of 3 days. No residue remained after 3 days.
2. *Paint samples*. A combination of pulverization (flat-finish paint) and chopping with a ceramic blade (glossy paint) sufficiently homogenized the paint samples. Subsamples of 20–100 mg of the homogenized paint were digested in an automated laboratory microwave digestion system (Milestone Incorporated., Sorisole, Italy) by using an Ethos Pro-24 rotor in a mix of high-purity acids (8 mL HNO_3, 2 mL HCl, 2.5 mL HF, and 1.5 mL H_2O_2). The microwave program consisted of a 20-min ramp to 10 min at 110 °C and a 60-min ramp to 20 min at 205 °C, at a constant power of 1200 W. After two microwave cycles, a certain amount of green solid, presumptively chromium(III) oxide, remained in the two paint samples; however, all forms of available lead should have been solubilized during this rigorous digestion [23, 24]. This solid was filtered out of the sample and retained for possible future analysis. We contend that if 2 cycles through the microwave procedure did not bring this minor solid component into solution, any Pb remaining in the solid should not be considered bio-available [25] and therefore not contributing to the isotopic signature of the blood.
3. *Tap water*. Concentration of Pb in the tap water was only 117 ng/L, so 250 mL of water was evaporated to dryness and taken up in 0.6 M HBr, before further purification, to have sufficient Pb for analysis using MC-ICPMS.
4. *Lead pipe*. Before any sectioning, scale deposits from the inside of the pipe were removed (without scratching the pipe, itself) and collected for analysis. Multiple thin washers were then sliced from one end of the pipe by using a band saw. Freshly exposed metal from the slices was cleaned of any remaining scale or metal shavings with 10 % HCl, and then multiple shavings were obtained by using a ceramic blade. A total of 20–40 mg of pipe shavings were digested similarly to the Pb bullet, 30 % HNO_3 for 3 days. The pipe scale (40–130 mg samples) was digested in a laboratory microwave with the same program and mixture of acids as the paint samples above (however, scale was only put through one complete cycle).

For all types of digestions and sample processing, procedural blanks and CRMs were also included in the analytical batch and treated similarly.

Aliquots of solutions from the bullet and paint digests were diluted and analyzed for total Pb by the inorganic metal section of the Wisconsin Occupational Health Laboratory at the WSLH by inductively coupled plasma-optical emission spectrometry, according to EPA methods 200.7 and SW846 6010B. WB, pipe, pipe scale, tap water, bullet, and paint digests were analyzed for total elemental composition (50 elements) on a single-collector high-resolution magnetic sector ICPMS instrument (HR-ICPMS), either an Element XR™ or Element 2™ (Thermo Fisher Scientific, Incorporated, Waltham, MA), in the clean laboratory of WSLH.

Reference Materials

Two WB reference materials were analyzed in this study to confirm method performance as follows: the CRM UTAK Metals™ in Whole Blood-Level 2 (product number 44522, UTAK Laboratories Incorporated, Valencia, CA) and Lead and Multielement Proficiency standard (LAMP) 1408, a Pb-enriched bovine WB used for interlaboratory quality assurance (WSLH Proficiency Testing Program, Madison, WI). These WB reference materials were processed in an analogous way to the WB patient samples.

A limited number of published studies process WB in a manner compatible with high-precision isotope ratio analysis on MC-ICPMS, and they often have certain recovery concerns such that a spike method needs to be applied [13]. In this study, we used both an unspiked and mixed-spike Pb analysis with WB CRMs to prove that our sample preparation approach, traditionally used for geologic samples because it results in least possible isotope fractionation, can be applied to WB. Additionally, National Institute of Standards and Technology (NIST) CRM 981 (certified Pb isotope values) and BCR-2 (CRM from the US Geological Survey, used in this case as a proxy for the pipe scale to provide an additional level of quality assurance for processing of this solid material) were independently processed in similar manners to both WB and the other samples (i.e., both oven or microwave digestion followed by the appropriate sample cleanup). The Element XR™ HR-ICPMS was used for determination of Pb recoveries and blank levels.

Sample Purification

As with the majority of MC-ICPMS applications, precision and accuracy of the isotope ratio measurement are severely degraded by sample matrix elements, and extensive sample cleanup (i.e., matrix removal) is required to achieve the highest possible isotope ratio precision. Both the inorganic and organic matrix components are problematic, causing variable ionization of the target element, isobaric and molecular interferences, and sample uptake concerns. WB, in particular, exposes the instrument to a highly undesirable matrix. All samples for Pb stable isotope analysis were evaporated to dryness on hot plates post-digestion, and Pb was converted to the bromide form (by two additions and successive

evaporations of 250 μL of HBr). Samples were then reconstituted in 0.6 M HBr and passed through an anion exchange resin (AG® 1 × 8 100–200 mesh, chloride form, Bio-Rad Laboratories, Incorporated, Hercules, CA). Before use, the resin was preconditioned in bulk with 1 M HCl and rinsed multiple times with ultrapure water until a neutral pH was reattained. Column loading (resin + H_2O), conditioning (H_2O and 0.6 M HBr), sample load volume (0.6 M HBr), and subsequent collection of the Pb cut (6 M HCl) were all scaled according to the amount of Pb in the sample. This protocol follows the column exchange method recommended by Johnson and Thompson [3] for geological samples. Pb cuts were spiked with H_2O_2 to reduce organic carbon levels, evaporated to dryness, reconstituted in HNO_3 + H_2O_2, re-evaporated, and reconstituted in HNO_3 to a volume of 0.5–1.0 mL before isotope analysis. The purified extracts were further diluted for MC-ICPMS analysis.

Isotope Analysis

Pb stable isotope ratios were measured by using a Neptune Plus™ MC-ICPMS (Thermo Fisher Scientific, Incorporated, Waltham, MA) in static mode with the Faraday cup configuration and other general parameters listed in Table 1. The ^{202}Hg was monitored to correct for the possible presence of ^{204}Hg, an isobaric interference for ^{204}Pb. Instrumental mass discrimination corrections were based on NIST 981 bracketing standards by using the exponential law and use of $^{205}Tl/^{203}Tl$ as an internal standard; all samples were spiked with Tl (High-Purity Standards, Incorporated, Charleston, SC) such that Pb/Tl = 5:1 in the final analyte solution [16, 17, 26]. All samples, reference materials, and bracketing standards were blank corrected. Data acquisition was structured as five blocks of 25 cycles/block (125 total ratio measurements/sample) with one integration/cycle. Two different MC-ICPMS front-end inlet configurations were used, including an Apex HF-model desolvating nebulizer (Elemental Scientific, Omaha, NB) and a standard quartz cyclonic spray chamber; both were used in self-aspirating mode. Target Pb and Tl concentrations in the final analyte solutions were 10 parts per billion (ppb) Pb + 2 ppb Tl with the Apex and 40 ppb Pb + 8 ppb Tl with the cyclonic spray chamber. The samples, reference materials, and bracketing standards were prepared such that the Pb and Tl concentrations were as consistent as possible and had a final matrix composition of 2 % *v*/*v* HNO_3. This concentration of HNO_3 was also determined to be sufficient for effective removal of residual sample Pb from the system after 4 min of rinsing.

Table 1 Neptune Plus™ Faraday cup configuration and selected frontend parameters

Detector[a]	Mass
L3	^{202}Hg
L2	^{203}Tl
L1	^{204}Pb
Center or axial	^{205}Tl
H1	^{206}Pb
H2	^{207}Pb
H3	^{208}Pb
Frontend parameters	
Resolution:	Low
Cool gas (L/min):	16
Auxiliary gas (L/min):	1.4
Typical sample gas flow (L/min)	
With quartz cyclonic spray chamber:	1.18–1.23
With Apex-HF:	1.16–1.19
Apex-HF additional gas:	Ar
Apex-HF additional gas flow (L/min):	0.13–0.19
PFA nebulizer uptake rates (μL/min):	105–143
Typical forward Power (W):	1210–1227

[a] *L* low-mass side, *H* high-mass side

Results

Total Pb

Total Pb concentrations for the human WB (after chelation), paint, bullet, pipe, scale, and tap water samples are presented in Table 2. Post-digestion recoveries of Pb for the UTAK Metals™ Level 2 and LAMP 1408 were >93 %.

Table 2 Total lead (Pb) in human whole blood (WB) after chelation[a] and source materials

Sample	Sampling date	[Pb][b]	Method[c]
Adult WB no. 1	August 28, 2015	66.5 μg/dL	1
Adult WB no. 2	August 29, 2015	67.5 μg/dL	1
Adult WB no. 3	August 30, 2015	65.5 μg/dL	1
Adult WB no. 4	September 20, 2015	77.4 μg/dL	1
Child WB no. 1	August 26, 2015	10 μg/dL	1
Child WB no. 2	August 26, 2015	10 μg/dL	1
Paint A (window)	September 9, 2015	2.7 %	2
Paint B (door)	September 9, 2015	3.1 %	2
Bullet	August 7, 2015	95 %	2
Pipe	March 9, 2016	>98 %	3
Scale from Pb pipe	March 9, 2016	3.8 %	3
Tap water	March 9, 2016	0.117 μg/L	3

[a] The adult received chelation therapy; the child did not

[b] Reference level of Pb in human WB is 5 μg/dL [19]

[c] 1, Parsons and Slavin [22]; 2, EPA methods 200.7 and SW846 6010B; 3, High-resolution inductively coupled plasma mass spectrometry, WSLH trace element clean laboratory

Considering the reference level of Pb in human blood, 5 μg/dL [19], the allowable amount of Pb in house paint (<0.06 %, US Consumer Product Safety Commission), and the maximum contaminant level goal of zero Pb in drinking water (US EPA), the Pb concentrations measured in all of these samples are of concern to human health. Recoveries for those same WB reference materials post-processing (including digestion, conversions or evaporations, column separation, and post-column conversions and evaporations) were >81 %. Concentration results for other select elements in the blood samples and Pb source materials can be found in Table 1 of the supplemental data.

Pb Isotopes

WB, Paint, Bullet, Pipe, and Tap Water

Our high-precision stable isotope analyses show distinct Pb fingerprints for the bullet, two different paint samples, Pb pipe, tap water, and child's WB (Fig. 1, Table 3). External precision (reproducibility of measurements performed on separate analytical runs) values for any of the blood or source samples do not exceed 1 ‰, 2σ (Table 3, ‰ = parts per thousand). Comparison with the precision of <0.4–5.8 ‰ reported by Chillrud et al. [13] gives an initial indication that complete digestion followed by a more thorough column cleanup procedure (rather than Fe co-precipitation only) is certainly worth the effort, if not necessary, when the application of interest requires a higher level of precision. The different blood draws are isotopically similar for each of the patients, indicating that we have captured a robust snapshot of the Pb pool in these subjects. Even if all results from the same patient do not fall within error of one another, they are still similar, compared with the vastly different (isotopically speaking) sources considered. The isotopic fingerprint of the adult's WB is most similar to that of the bullet (Figs. 1 and 2) and nearly indistinguishable. Pb isotopic fingerprints of the pipe and scale from the inside of the pipe and the pipe itself are also indistinguishable from one another (Figs. 1 and 2). Procedural and analytical duplicates analyzed on a select number of the samples have a mean relative difference <1.2 ‰.

CRMs and Spiked WB Reference Materials

Pb isotope results for NIST 981 carried through the complete processing protocol (n = 6, with both digestion methods represented) are in good agreement with published values [26, 27], indicating minimal isotope fractionation during sample processing, with ratios matching nearly within precision error of published values (match within <0.4 ‰, with external precision of <0.3 ‰).

On average, spike-corrected results agree with unspiked data within 0.34 % (0.001–1.7 %, depending on ratio and analyte UTAK or LAMP 1408). Spike-corrected versus unspiked $^{206}Pb/^{204}Pb$ match within 0.79 and 0.015 %, and $^{207}Pb/^{204}Pb$ match within 0.041 and 0.023 % (UTAK and LAMP 1408, respectively). This agreement is vital, given that we have demonstrated that these unspiked methods, previously reserved for geologic or inorganic samples, can be applied to blood if substantial care is taken to avoid contamination and fractionation. We were unable to determine if any discrepancies were attributable to fractionation or the effect of spike concentration relative to native Pb [2, 28] without extensive further study.

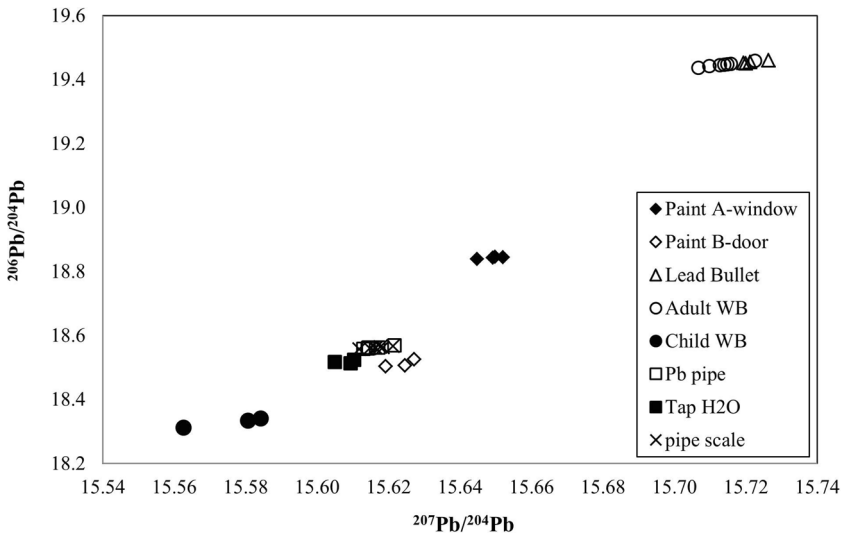

Fig. 1 Two-dimensional isotope ratio plot ($^{207}Pb/^{204}Pb$ versus $^{206}Pb/^{204}Pb$) for all samples in this study, including adult and child whole blood (WB) and multiple lead (Pb) sources. At this scale, all 2× standard error bars are smaller than the symbols

Table 3 Averaged lead (Pb) isotope ratios in human whole blood (WB) and source materials

	Adult WB	Child WB	Paint A (window)	Paint B (door)	Bullet	Pb pipe	Pipe scale	Tap water
n	7	3	4	3	4	6	7	3
$^{208}Pb/^{207}Pb$	2.46961	2.43303	2.45718	2.44640	2.46979	2.45048	2.45049	2.45553
± 2× Std. Err.	0.00002	0.00003	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002
2σ ext. prec. (‰)	0.020	0.043	0.024	0.206	0.034	0.018	0.033	0.142
$^{206}Pb/^{207}Pb$	1.23743	1.17661	1.20412	1.18484	1.23738	1.18856	1.18854	1.18633
± 2× Std. Err.	0.00001	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00002
2σ ext. prec. (‰)	0.017	0.142	0.115	0.507	0.041	0.036	0.031	0.271
$^{208}Pb/^{206}Pb$	1.99574	2.06784	2.04066	2.06477	1.99597	2.06172	2.06176	2.06987
± 2× Std. Err.	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00003
2σ ext. prec. (‰)	0.010	0.151	0.127	0.320	0.022	0.040	0.031	0.152
$^{208}Pb/^{204}Pb$	38.8071	37.8972	38.4515	38.2212	38.8289	38.2664	38.2686	38.3268
± 2× Std. Err.	0.0014	0.0035	0.0012	0.0013	0.0019	0.0013	0.0013	0.0018
2σ ext. prec. (‰)	0.253	0.850	0.214	0.481	0.216	0.175	0.152	0.204
$^{207}Pb/^{204}Pb$	15.7139	15.5759	15.6489	15.6236	15.7217	15.6159	15.6167	15.6083
± 2× Std. Err.	0.0005	0.0014	0.0005	0.0005	0.0008	0.0005	0.0005	0.0007
2σ ext. prec. (‰)	0.239	0.859	0.193	0.301	0.202	0.167	0.144	0.210
$^{206}Pb/^{204}Pb$	19.4449	18.3269	18.8429	18.5114	19.4538	18.5604	18.5611	18.5165
± 2× Std. Err.	0.0006	0.0017	0.0006	0.0005	0.0009	0.0006	0.0006	0.0009
2σ ext. prec. (‰)	0.257	0.948	0.178	0.734	0.205	0.164	0.136	0.336
$^{207}Pb/^{206}Pb$	0.808124	0.849901	0.830482	0.843998	0.808161	0.841352	0.841367	0.842936
± 2× Std. Err.	0.000006	0.000012	0.000009	0.000007	0.000007	0.000007	0.000007	0.000011
2σ ext. prec. (‰)	0.017	0.142	0.115	0.507	0.041	0.036	0.031	0.271

n = number of isotope analyses; this includes analytical duplicates and procedural duplicates. Blood samples from the same patient were isotopically similar at our level of precision

Blanks

Digestion, column, and full procedural blanks were monitored throughout the project and ranged from 37 to 269 pg Pb (Table 4). This range falls within previous measurements of Pb in blanks prepared in TECL by using similar methods, with the microwave digestion step contributing the most Pb. The typical procedural blank (having gone through full sample processing) represents <1 % of the Pb in the prepared samples.

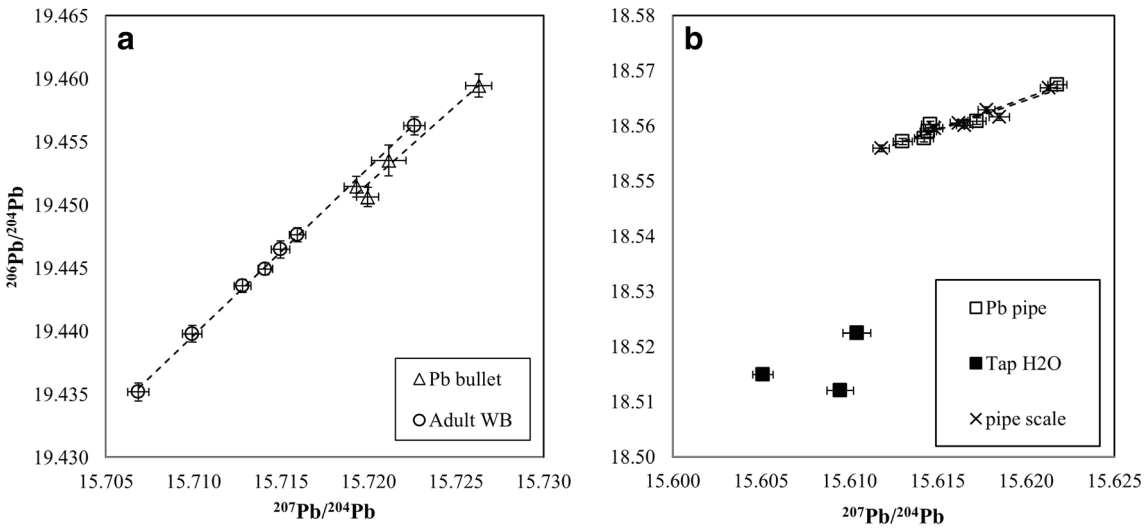

Fig. 2 *Zoomed* portions of Fig. 1. **a** Isotope plot exhibiting the correlation between the lead (Pb) isotope fingerprint of the adult whole blood (WB) and the lead bullet fragment. A regression including both the adult WB and the bullet gives an R^2 value of 0.992. **b** The tap water and Pb pipe or pipe scale have isotopically distinct Pb signatures. *Error bars* are two standard errors

Table 4 Total lead (Pb) values for different types of blanks analyzed during method development and subsequent sample preparation and analysis

Type of blank	Handling description	Number	Pb (pg)[a]	±
Procedural blank	Oven digestion + column separation	3	42	1.3
Procedural blank	Microwave digestion + column separation	2	269	0.4
Column blank	Column separation only	3	37	1.1

[a] Measured by high-resolution inductively coupled plasma mass spectrometry, WSLH trace element clean laboratory; results are averaged

Discussion

Lead Exposure Sources: Adult

When we consider the five potential Pb sources experimentally investigated (paint A, paint B, bullet, pipe, and tap water; Fig. 1), the adult's WB is isotopically most similar to the bullet ($R^2 = 0.992$, Fig. 2a). The difference in isotopic fingerprints is limited (0.05–0.56 ‰, depending on the isotope ratio), but is a statistically significant difference ($P \leq 0.0001$–0.038, two-tailed t test) only because of the use of ultrahigh-precision MC-ICPMS tools. Given the extremely elevated BLLs, with an isotopic signature approximating that of the bullet, the bullet is likely the source of the elevated BLL. Whether the measured minute differences in isotopic ratios are an analytical artifact attributable to minor fractionation during preparation or analysis for these two different matrices (WB versus Pb bullet) or if they are the result of some isotopic fractionation process that might be occurring within the body is difficult to ascertain without additional study, because literature concerning Pb isotope fractionation or partitioning within the human body is lacking. The initial studies of isotope fractionation during processing that we performed by using CRMs indicate that artefactual isotope fractionation was vanishingly small (NIST 981 results match published results within <0.4 ‰). Although the weight of evidence in this study overwhelmingly indicates that the bullet was the elevated BLL source, given the limited but substantial difference in isotopic fingerprints, prudence dictates that any conclusions made with respect to Pb isotopes in human clinical samples should come with the caveat that Pb isotope fractionation within the human body is a nearly unexplored field and until the scope and magnitude of potential fractionation processes are defined, source attribution (and source exclusion) of lead will ultimately be limited by these unknowns. For example, the extent of lead mobilization from the bones is a known function of stress in the body (e.g., sepsis, shock, healing of a broken bone, pregnancy, and lactation) [20, 29, 30] and that Pb movement in or out of bones [31] likely fractionates Pb isotopes to a certain extent [32]. However, in this particular case, a plausible explanation exists that physiologic isotopic fractionation was minimal given the mode of release and exposure to the acute dose of lead (i.e., possible disruption of the bullet fragments attributable to the car accident) and subsequent release of Pb from the soft-tissue cyst (presumably where partial dissolution of the bullet might have occurred), thus leading to symptoms of elevated blood Pb levels.

Of further concern for the patient is the substantial potential for continuing release of Pb to the blood stream from bullet fragments. Considering the ~5 L of blood in an adult male, this patient would have had 10 mg of Pb in his blood before chelation (initial BLL >200 μg/dL). That pool of Pb represents just <0.3 % of the mass of the surgically removed bullet fragment (3700 mg fragment removed). Assuming similar dissolution environments and the slow excretion of Pb from the body, this indicates that without intervention that fragment alone could have continued to poison the patient's blood for decades and that the remaining bullet fragments retained in the thigh of the patient should still be considered of substantial concern.

Comparison of the bullet Pb isotope results with those published values from bullets from around the world reveals that of the brands of bullets sampled by Sjåstad et al. [11], the bullet in this study is most isotopically similar to Remington Target and Winchester Super-XII bullets, both of which are from the USA. However, we cannot make any definitive conclusions beyond stating that the bullet in this study has a similar Pb isotopic fingerprint to that of those particular lots or boxes from two different American bullet brands sampled by Sjåstad et al. [11] (see Fig. S1 of the Supplemental Material). At the very least, these observations might exclude certain brands (or at least certain lots) of bullets as forensic candidates.

Lead Exposure Sources: Child

Of the sources investigated, no single source, including paints and tap water from within the most recent residence of the child, stands out as most likely to be the cause of the child's elevated BLL. Records indicate that the child resided in the apartment building for ~1 year (February 2015–March 2016). More definitive source attribution will require further assessment of the history of the child's living accommodations and potential for additional exposures. Since moving out of the residence in March 2016, the child's BLL was measured at 2 μg/dL, a significant decline from the 10 μg/dL reported previously, without any treatment, further indicating that the source of Pb was somewhere in or around the residence and is particularly concerning for any children that may reside there presently.

Considering recent changes in Pb exposure guidelines in children, e.g., lowering the BLL reference level in children from 10 to 5 μg/dL [33] and widespread concern regarding US urban children consuming high levels of Pb in their drinking water,

investigating tap water and a neighborhood Pb service line pipe as a substantial source of Pb to the child is practical. Given the low levels of Pb in the tap water in this case, this exposure route is likely minimal, but our methods clearly demonstrate that fingerprinting drinking water sources is possible even at Pb concentrations well below drinking water action levels (with the necessity of preparing these samples in a clean laboratory setting where total blanks contain <300 pg Pb).

An average-sized child aged 2 years will consume up to ~0.9 L of water/day (~330 L/year) [34]. Even if the child consumed all fluids in the form of drinking water, the child would still consume, at most, ~40 μg Pb during the course of a year from the analyzed tap water. Assuming a worst case scenario where all Pb was retained, the child would still have to have been exposed to another Pb source to reach the observed BLL. The measured isotopic fingerprints can help narrow the search for the unidentified source of Pb. Regardless of whether the tap water is taken into account as a minor point source, a theoretical mixing model would require at least one other major Pb source with a less radiogenic signature (less ^{204}Pb) than the child's blood if one were to consider the paints (both or individually) as the other end-member (point source). Certain information concerning the relative contribution of the potential end-members would be needed to test the validity of a two-source or multisource component isotope mixing model.

Multiple explanations exist for why the tap water has a different isotopic signature than the pipe or pipe scale. Most obviously, Milwaukee Water Works is unlikely to have used Pb pipes from the same source for buildings in 1954 as in 1916; unfortunately, the 1916 Pb pipe was the only sample from that neighborhood available and will have to serve as a proxy until the Pb service laterals are eventually replaced in the 1954 apartment building. Additionally, Cheng and Foland [6] observed that domestic water samples often have a different isotopic signature than the Pb service pipes and that the Pb signature of the water tends to match more closely with that of the joint solder used on the pipes within the house rather than the pipes themselves. First-flush tap water is assumed to have been sitting in domestic pipes overnight, not in the service lines outside the home, so the isotopic signature should reflect the contribution of joint solders within the home. Moreover, they point out that scale deposit inside the pipe can reduce contact between the water and the pipe. A combination of these factors likely offers a possible explanation as to why the tap water Pb signature does not match the signature of the pipe in this study. The isotopic fingerprint of this pipe (circa 1916) does not match those of the pipes measured by Cheng and Foland [6] (who measured two, distinct Pb isotope fingerprints from Pb service lines installed before 1940 in urban Columbus, Ohio (Fig. S2 of the Supplemental Material).

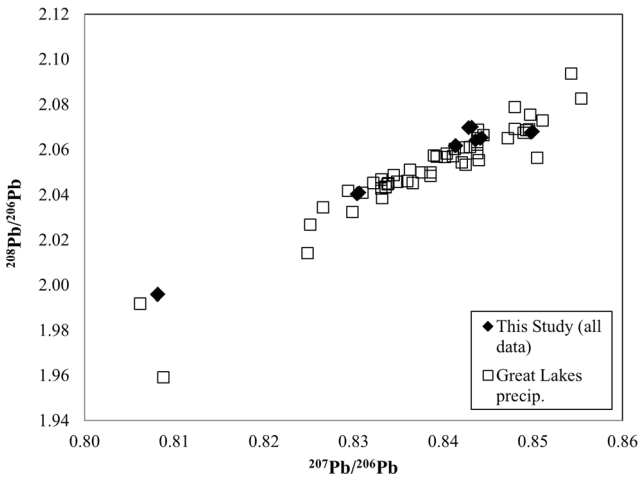

Fig. 3 Data comparison; all data collected in this study compared with lead isotopic ranges reported by Sherman et al. [37] in precipitation collected east of Wisconsin (Michigan, Ohio, and Vermont), at urban and rural collection sites. At this scale, the symbols are larger than the 2× standard error bars (this study) or 1× standard deviation bars, reported by Sherman et al. [37]

Environmental Context of Measured Pb Isotope Ratios

Interestingly, both the child and adult blood Pb isotope results registered below and above, respectively, the "modern environmental Pb" range for the eastern and central USA established by two studies based on blood and urine of children and adults with nonelevated BLL [8, 9]. For the adult, this reflects the dominant influence of the bullet-sourced Pb with a substantial disparate isotope signature. As for the child, a local environmental Pb source contribution that is yet unidentified affecting the integrated Pb isotopic fingerprint of the blood is likely.

All Pb isotope results in this study fall within expected Pb source ranges in the USA, e.g., the isotopic Pb range within US geologic end-members, averaged urban aerosols, leaded gasoline, and coal [4, 9, 35, 36]. Moreover, results fall within reported Pb isotope ranges of precipitation collected in the Great Lakes region whose isotopic fingerprint has been partially attributed to point sources in Wisconsin, including certain industrial sources in the Milwaukee region [37], as shown in Fig. 3.

Conclusions

Our data overwhelmingly indicate that the bullet is the source of elevated blood Pb levels for the adult subject, which is a disconcerting discovery, because bullets embedded in soft tissue had previously not been considered a risk factor for elevated blood Pb levels. However, given Pb sources evaluated, we cannot provide a complete accounting of the cause(s) of elevated BLL in the child's case. The water and paint might have contributed to the overall Pb signature of the child's blood while living at that residence, but those contributions

might not tell the whole story. Indeed, this case study illustrates the utility and power of source tracking by Pb isotope fingerprinting (i.e., the bullet as an elevated blood Pb level source for the adult) and demonstrates that comprehensive source tracking can be more challenging than first imagined (i.e., source of elevated BLL for the child). Given contrasts measured in this study in Pb fingerprints among the paints and tap water, these methods will likely assist in the visible and extremely important concern of Pb sourcing from contaminated tap water. An improved understanding of Pb isotope fractionation that might occur in the human body and applications of ever-improving MC-ICPMS capabilities will only benefit the field of public health in cases like this.

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Compliance with Ethical Standards Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Conflict of Interest The authors declare they have no conflict of interest.

Ethical Approval For this type of study, formal consent is not required.

References

1. Dewan N, Majestic BJ, Ketterer ME et al (2015) Stable isotopes of lead and strontium as tracers of sources of airborne particulate matter in Kyrgyzstan. Atmos Environ 120:438–446
2. Galer S (1999) Optimal double and triple spiking for high precision lead isotopic measurement. Chem Geol 57:255–274
3. Johnson C, Thompson R (1991) Isotopic composition of Oligocene mafic volcanic rocks in the northern Rio Grande Rift: evidence for contributions of ancient intraplate and subduction magmatism to evolution of the lithosphere. J Geophys Res 96:13,593–13,608
4. Komarek M, Ettler V, Chrastny V, Mihaljevic M (2008) Lead isotopes in environmental sciences: a review. Environ Int 34:562–577
5. Chaudhary-Webb M, Paschal DC, Romieu I et al (2003) Determining lead sources in Mexico using the lead isotope ratio. Salud Publica Mex 45:S183–S188
6. Cheng Z, Foland KA (2005) Lead isotopes in tap water: implications for Pb sources within a municipal water supply system. Appl Geochem 20:353–365
7. Fillion M, Blais JM, Yumvihoze E et al (2014) Identification of environmental sources of lead exposure in Nunavut (Canada) using stable isotope analyses. Environ Int 71:63–73
8. Graziano JH, Blum CB, Lolacono NJ et al (1996) A human *in vivo* model for the determination of lead bioavailability using stable isotope dilution. Environ Health Perspect 104:176–179
9. Jaeger RJ, Weiss AL, Manton WI (1998) Isotopic ratio analysis in residential lead-based paint and associated surficial dust. J Toxicol Clin Toxicol 36:691–703
10. Okuda T, Schauer JJ, Shafer MM (2014) Improved methods for elemental analysis of atmospheric aerosols for evaluating human health impacts of aerosols in East Asia. Atmos Environ 97:552–555
11. Sjåstad KE, Simonsen SL, Andersen TH (2014) Lead isotope ratios for bullets, a descriptive approach for investigative purposes and a new method for sampling of bullet lead. Forensic Sci Int 244:7–15
12. Sjåstad KE, Lucy D, Andersen T (2016) Lead isotope ratios for bullets, forensic evaluation in a Bayesian paradigm. Talanta 146: 62–70
13. Chillrud SN, Hemming NG, Ross JM, Wallace S, LoIacono N (2005) A rapid and precise procedure for Pb isotopes in whole blood by Fe co-precipitation and MC-ICPMS analysis. Appl Geochem 20:807–813
14. Gwiazda RH, Smith DR (2000) Lead isotopes as a supplementary tool in the routine evaluation of household lead hazards. Environ Health Perspect 108:1091–1097
15. Tsuji LJS, Wainman BC, Martin ID et al (2008) Lead shot contribution to blood lead of First Nations people: the use of lead isotopes to identify source of exposure. Sci Total Environ 405:180–185
16. Kuritani T, Nakamura E (2002) Precise isotope analysis of nanogram-level Pb for natural rock samples without use of double spikes. Chem Geol 186:31–43
17. Tanimizu M, Ishikawa T (2006) Development of rapid and precise Pb isotope analytical techniques using MC-ICP-MS and new results for the GSJ rock reference samples. Geochem J 40:121–133
18. Weiss D, Tomasallo CD, Meiman JG, et al. Severe lead poisoning due to retained bullet in soft tissue. Brit Med J manuscript in review
19. Center for Disease Control and Prevention (2015) Adult blood lead epidemiology andsurveillance (ABLES), report of the National Institute for Occupational Safety and Health (NIOSH). Available at: http://www.cdc.gov/niosh/topics/ables/description.html. Accessed August 25, 2016
20. McQuirter JL, Rothenberg SJ, Dinkins GA et al (2001) The effects of retained lead bullets on body lead burden. J Trauma 50:892–899
21. McQuirter JL, Rothenberg SJ, Dinkins GA et al (2004) Change in blood lead concentration up to 1 year after a gunshot wound with a retained bullet. Am J Epidemiol 159:683–692
22. Parsons PJ, Slavin W (1993) A rapid Zeeman graphite furnace atomic absorption spectrometric method for the determination of lead in blood. Spectrochim Acta B At Spectrosc 48:925–939
23. Viczián M, Lásztity A, Barnes RM (1990) Identification of potential environmental sources of childhood lead poisoning by inductively coupled plasma mass spectrometry, verification and case studies. J Anal Atom Spec 5:293–300
24. Silva FLF, Duarte TAO, Melo LS et al (2016) Development of a wet digestion method for paints for the determination of metals and metalloids using inductively coupled plasma optical emission spectrometry. Talanta 146:188–194
25. Deshommes E, Tardif R, Edwards M et al (2012) Experimental determination of the oral bioavailability and bioaccessibility of lead particles. Chem Central 6:138
26. Platzner I, Ehrlich S, Halicz L (2001) Isotope-ratio measurements of lead in NIST standard reference materials by multiple-collector inductively coupled plasma mass spectrometry. Fresenius J Anal Chem 370:624–628
27. Hart S, Ball L (2004) Isotopic homogeneity of the SRM 981 Pb isotopic standard. Woods Hole Oceanographic Institution, WHOI Plasma Facility Open File Technical Report 12:1–3
28. Hirata T (1996) Lead isotopic analyses of NIST standard reference materials using multiple collector inductively coupled plasma mass

spectrometry coupled with a modified external correction method for mass discrimination effect. Analyst 121:1407–1411
29. Gulson BL, Jameson CW, Mahaffey KJ et al (1997) Pregnancy increases mobilization of lead from maternal skeleton. J Lab Clin Med 130:51–62
30. Gulson BL, Mahaffey KR, Jameson CW et al (1998) Mobilization of lead from the skeleton during the postnatal period is larger than during pregnancy. J Lab Clin Med 131:324–329
31. Skulan J, Bullen T, Anbar AD et al (2007) Natural calcium isotopic composition of urine as a marker of bone mineral balance. Clin Chem 53:1155–1158
32. Gulson BL, Taylor A, Eisman J (2016) Bone remodeling during pregnancy and post-partum assessed by metal lead levels and isotopic concentrations. Bone 89:40–51
33. Center for Disease Control and Prevention (2012) Low level lead exposure harms children: a renewed call for primary prevention, report of the Advisory Committee on Childhood Lead Poisoning Prevention. Available at: www.cdc.gov/nceh/lead/acclpp/final_document_030712.pdf. Accessed July 5, 2016
34. U.S. Institute of Medicine (2005) Panel on dietary reference intakes for electrolytes and water: dietary reference intakes for water, potassium, sodium, chloride, and sulfate. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board. The National Academies Press, Washington, DC
35. Flegal AR, Nriagu JO, Niemeyer S, Coale KH (1989) Isotope tracers of lead contamination in the Great Lakes. Nature 339:455–458
36. Ketterer ME, Lowry JH, Simon J Jr et al (2001) Lead isotopic and chalcophile element compositions in the environment near a zinc smelting-secondary zinc recovery facility, Palmerton, Pennsylvania, USA. Appl Geochem 16:207–229
37. Sherman LS, Blum JD, Dvonch T et al (2015) The use of Pb, Sr, and Hg, isotopes in Great Lakes precipitation as a tool for pollution source attribution. Sci Total Environment 502:362–374