Validation of X-Ray Fluorescence-Measured Swine Femur Lead Against Atomic Absorption Spectrometry

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The aim of this study was to apply the technique of ^{109}Cd-based K-shell X-ray fluorescence (XRF) bone lead measurements to swine femurs and to validate the concentrations obtained therefrom against an independent chemical measurement of bone lead: atomic absorption spectrometry (AAS). The femurs ranged in lead concentration from 1.0 to 24.5 μg of lead per gram of ashed bone, as measured by AAS. On average, XRF overestimated AAS-measured femur lead by 2.6 μg/g [95% confidence interval (CI), 1.1–4.0 μg/g], approximately 2 μg/g poorer than that observed in studies of human tibiae. Measurements of swine femur and, by extension, of nonhuman bones may require adjustment of the XRF spectrum peak extraction method. *Key words*: atomic absorption, lead poisoning, spectrometry, spectrophotometry, X-ray fluorescence. *Environ Health Perspect* 109:1115–1119 (2001). [Online 19 October 2001]
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Exposure to lead is monitored most commonly by measuring blood lead levels, and in the United States the criteria for lead poisoning and lead toxicity are based on blood lead as a standard. However, the biologic residence time of lead in blood is approximately 36 days (*1*), and it is therefore an indicator only of recent lead exposure. Moreover, the concentration of lead in blood is a composite index that reflects the equilibrium among current exogenous exposure, excretory loss, and the movement of lead between bone and other deep compartments (endogenous exposure). The relative contribution to the blood lead level of each of these sources varies with the levels of current exposure and body burden.

Lead is stored in the human body predominantly in calcified tissues; 90–95% of the total lead burden is contained within bone in nonoccupationally exposed adults (*2,3*). The turnover rate of lead in bone is slow; quantitative estimates of the characteristic residence time vary, but there is a consensus that it is on the order of years or even decades (*1,4–6*). Throughout childhood and most of adult life, lead exposure from both environmental and occupational sources increases lead concentration within calcified tissue. Bone lead content thus reflects integrated or cumulative lead exposure (*7*).

Bone lead can be measured noninvasively and *in vivo* by the technique of ^{109}Cd photon-induced K-shell energy-dispersive X-ray fluorescence (XRF) (*6,8,9*). The 88.034 keV gamma rays emitted by a ^{109}Cd source are used to excite the lead atoms contained in bone. The lead atoms in the bone subsequently de-excite and may thereby emit X rays of energy specific to lead. The lead X rays are recorded by a radiation detector and, when compared with calibration data, yield a measure of the lead content of the bone. Although the technique delivers a radiation dose to the subject, the radiation dose and consequent risk arising from an XRF bone lead measurement are very small for all age groups, including children (*10*).

Three previous reports in the literature compare ^{109}Cd KXRF to independent chemical measurements of lead in bone in humans. Somervaille et al. (*11*) analyzed 30 bone samples and found no evidence of a statistically significant difference between XRF and atomic absorption spectrometry (AAS). Hu et al. (*12*) analyzed eight locations from three legs and reported a correlation coefficient of 0.98 between XRF and AAS. Aro et al. (*13*), using inductively coupled plasma mass spectrometry, measured eight cadaver legs and also reported the agreement with XRF as correlation coefficients.

Herein we report results of a comparison between XRF and AAS measurements of the lead concentration in 44 swine femurs. Validation of our XRF method using animal bones interests us because animals that have undergone controlled dosing with lead may serve in the future as a source of calibration, validation, or standard reference materials for human XRF measurements.

Materials and Methods

All procedures performed on animals followed the NIH *Guide for the Care and Use of Laboratory Animals* (*14*), and were approved by the Institutional Animal Care and Use Committee of University of Missouri-Columbia. The femurs measured for this study came from the University of Missouri-Columbia study of the uptake and retention of lead in swine (*15*). The animals were dosed with 75, 225, and 675 μg Pb/kg body weight/day for 15 days with lead-contaminated soil.

XRF measurements. XRF was performed at the mid-shaft of bare left femurs using a spot source measurement system (*8*). The right femurs were destructively analyzed via AAS before the collaboration started; only the left femurs remained. The XRF measurement system consisted of a 2,000 mm^2 intrinsic germanium detector (Canberra model GL2020R; Canberra Industries, Meriden, CT, USA), amplifier (Canberra model 2024), 450 MHz Wilkinson analog-to-digital converter (Canberra model 8706), and multichannel analyzer (Canberra model S100). The source activity was 0.6 GBq (16 mCi). The amplifier Gaussian shaping time was 1 μsec, which gave a full-width at half-maximum (FWHM) of approximately 700 eV for the 88.034 keV peak from elastically scattered ^{109}Cd γ-rays for both calibration and bone spectra. Spectra were acquired for half-hour (true time).

For the analysis of spectra, a nonpolynomial mathematic function (the model) is fitted to the pulse-height distribution. The model used consists of one or more exponentials that represent the shape of the spectral background, Gaussian functions that represent the X-ray peaks, and a step function under each peak. The parameters of the mathematic function are then adjusted to give the best fit, as measured by the χ^2 per degree of freedom. The method of parameter adjustment used is credited to Marquardt (*16*). Curve fitting is conducted with a computer program written in Fortran, the essence of which has been described by Bevington (*17*). A second program produces the concentration of lead in bone and an estimate of the statistical uncertainty (one standard deviation) therein via comparison

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to the lead signal obtained from a set of 10 lead-acetate–doped plaster-of-paris calibration standards (*9*). The calibration standards ranged in lead concentration from 0 to 163 μg/g plaster-of-paris, equivalent to 238 μg/g bone mineral (*18*). The lead concentrations of the plaster calibration standards were not verified via a chemical method (e.g., AAS). We used the lead $K\alpha_1$ and $K\beta_1$ X-ray peaks (International Union of Pure and Applied Chemistry notation K-L_3 and K-M_3, respectively) to calculate estimates of the femur lead concentration ($K\alpha_1$[Pb] and $K\beta_1$[Pb], respectively), which we then combined (*9,19*) into an overall estimate (XRF[Pb]).

The measurement uncertainty, calculated using these algorithms, is not identical to the standard deviation of repeated measurements (*20*). Nevertheless, the measurement uncertainty is still of interest because it is a measure of the statistical uncertainty in the estimate of an individual lead concentration.

The lowest XRF[Pb] recorded was negative. Kim et al. (*21,22*) have examined how negative XRF[Pb] results can be obtained from measurements of low lead concentration bones because of XRF measurement error, and how retention of all the data "makes better use of the data for a population in epidemiologic studies" (*22*). All XRF[Pb] were therefore retained for the present study.

The International Union of Pure and Applied Chemistry lower limit of detection (three times the standard deviation of the predicted concentrations obtained from repeated measurements of a low-concentration sample) for measurements of a plaster-of-paris calibration phantom of nominal zero lead concentration, acquired over the course of the femur measurements, was 2.5 μg Pb per gram of plaster [equivalent to 3.6 μg Pb per gram of bone mineral (*18*)].

Calibration standards of nominal zero lead concentration showed no evidence of contamination of the standards themselves or of the room where bone lead measurements were performed [for a full discussion of the sources of contamination and how to correct for them, see Todd (*23*)].

AAS measurements. AAS measurements were made on the right femur. Soft tissue was flensed from the femurs with a knife. We dried the femurs in an oven for 4 hr before ashing. Graphite furnace AAS analysis was performed by a commercial laboratory (L.E.T. Inc, Columbia, MO), which received the bone samples in blinded fashion. The lab evaluated AAS detection limit for each sample and varied between 1 and 2 μg Pb (g ashed bone)$^{-1}$.

The commercial laboratory performed duplicate AAS analyses on a subset of the samples, all of which were within twice the sample detection limit. We used spiked samples to estimate sample recovery at 99.6% [standard deviation (SD) 10.5%]. We also ran an analytic check sample of 202 ng/mL, which was, on average, 100.3% of its certified value (SD 3.5%). Finally, we ran blank samples, all of which were < 1 ng/mL.

Statistical Analysis

We performed statistical analyses with SAS (SAS Institute Inc., Cary, NC). We examined box plots of AAS and XRF measurements for symmetry, normality, and any "extreme" outliers (an observation more than three interquartile ranges from the 25th or 75th percentiles). There were no extreme outliers, so we retained all data and included them in the results. We used Levene's test to assess the homogeneity of variances of the concentrations (i.e., the squares of the standard deviations of the results obtained from the members of a group) within the six technique × dose groups (i.e., groups of XRF and AAS concentrations for each of the three dose groups).

We assessed agreement between XRF and AAS across dose groups via mixed modeling (*24*), which accounted for the correlation between measurements made within the same pig. We tested nonlinear associations but found them not significant. We assessed parallelism of the "technique" (i.e., XRF and AAS) slopes (of lead concentration vs. dose) by testing the hypothesis that the interaction between technique and dose was equal to zero. When the interaction between technique and dose was not significantly different from zero, we estimated the difference between XRF and AAS from the model with parallel slopes (i.e., excluding the technique × dose interaction term).

To compare our data with those reported in the literature, we performed other analyses (which are less pertinent to measuring agreement): ordinary least squares unweighted regression between XRF and AAS, Pearson and Spearman (rank) correlation coefficients, and paired *t*-tests.

Results

The units of XRF results (μg of lead per gram of bone mineral) and AAS results (μg of lead per gram of ashed bone) are assumed to be identical; μg/g is therefore used for both hereafter.

The number of swine femurs measured from lead dose groups of 75, 225, and 675 μg/kg/day were 14, 15, and 15, respectively. Descriptive statistics for XRF[Pb] and AAS-measured femur lead concentrations (AAS[Pb]) are shown in Table 1. We compared AAS[Pb] to both the individual estimates of lead concentration given by $K\alpha_1$[Pb] and $K\beta_1$[Pb] and to the overall estimate, XRF[Pb]. The results of this analysis are shown in Table 2.

For each of the three XRF and AAS comparisons, the variances of the concentrations within the six technique × dose groups (two techniques in each of three dose groups) were not homogeneous (Levene's *p*-value < 0.012), but they were normally

Table 1. Descriptive statistics of 44 swine femur lead concentrations made via both XRF and AAS.

Method	Lead dose (μg/kg/day)	No.	Mean (μg/g)	SD (μg/g)	Minimum (μg/g)	Median (μg/g)	Maximum (μg/g)
AAS[Pb]	All	44	7.6	6.3	1.0	6.0	24.5
XRF[Pb]	All	44	10.2	6.2	–2.5	10.2	25.9
AAS[Pb]	75	14	2.0	0.6	1.0	2.2	2.8
XRF[Pb]	75	14	5.1	4.9	–2.5	4.4	12.0
AAS[Pb]	225	15	5.5	1.5	3.0	6.0	8.0
XRF[Pb]	225	15	9.6	4.1	5.2	8.8	18.8
AAS[Pb]	675	15	14.9	5.3	7.0	14.5	24.5
XRF[Pb]	675	15	15.4	4.7	7.1	15.1	25.9

Table 2. Mixed modeling analysis of estimates of femur lead concentration and lead dose.

Model	Estimate (SE)	*p*-Value
Comparing AAS[Pb] to $K\alpha_1$[Pb]		
Intercept (μg/g)	1.5575 (1.1753)	0.1923
Dose (μg/g per μg/kg/day)	0.0171 (0.0028)	< 0.0001
Technique (μg/g)	–0.9842 (1.4910)	0.5128
Technique × dose (μg/g per μg/kg/day)	0.0041 (0.0036)	0.2562
Comparing AAS[Pb] to $K\beta_1$[Pb]		
Intercept (μg/g)	8.1048 (1.0797)	< 0.0001
Dose (μg/g per μg/kg/day)	0.0149 (0.0026)	< 0.0001
Technique (μg/g)	–7.5315 (1.3681)	< 0.0001
Technique × dose (μg/g per μg/kg/day)	0.0063 (0.0033)	0.0602
Comparing AAS[Pb] to XRF[Pb]		
Intercept (μg/g)	4.8116 (0.9778)	< 0.0001
Dose (μg/g per μg/kg/day)	0.0162 (0.0023)	< 0.0001
Technique (μg/g)	–4.2383 (1.1841)	0.0009
Technique × dose (μg/g per μg/kg/day)	0.0051 (0.0028)	0.0807

distributed (Kolmogorov-Smirnov p-value > 0.10). The inhomogeneity of the variances was reduced but not removed by a natural logarithm transformation of the data, but there was no difference between the conclusions drawn from the transformed and untransformed data. The analyses of the untransformed data are presented for all aspects of the study both for ease of interpretation and because mixed modeling is moderately robust against unequal variances.

When $K\alpha_1$[Pb] is compared to AAS[Pb], mixed modeling (for which there were no influential observations) showed that dose was a significant factor, but that neither technique nor technique × dose was significant. The nonsignificance of technique and technique × dose indicates that the relationship between lead concentration (measured by $K\alpha_1$[Pb] and AAS[Pb]) and dose did not differ for the two techniques with regard to either the origins and the rates of change: The concentration-versus-dose lines were coincident; their slopes and intercepts were not significantly different. For AAS[Pb], the relation was:

AAS[Pb] = 0.5733 (± 1.1753; p = 0.6282)
+ [0.0212 (± 0.0028; p < 0.0001) × dose].

For $K\alpha_1$[Pb], the relation was:

$K\alpha_1$[Pb] = 1.5575 (± 1.1753; p = 0.1923)
+ [0.0171 (± 0.0028; p < 0.0001) × dose].

The difference between predicted AAS[Pb] and $K\alpha_1$[Pb] was 0.37 µg/g [SE 0.91 µg/g; p-value 0.68; 95% confidence limits (CL) –1.47 to 2.22 µg/g].

When comparing $K\beta_1$[Pb] and AAS[Pb], mixed modeling (for which there were no influential observations) showed that dose and technique were both significant factors and that technique × dose was also of borderline significance (p = 0.06). The marginal significance of technique × dose indicates that the relationships between lead concentration and dose were different for the two techniques: The concentration-versus-dose lines had slopes and intercepts that were significantly different. The relation for AAS[Pb] is given above. The relation for $K\beta_1$[Pb] was:

$K\beta_1$[Pb] = 8.1048 (± 1.0797; p < 0.0001)
+ [0.0149 (± 0.0026; p < 0.0001) × dose].

There was, therefore, a statistically significant difference between the lead concentrations obtained by the two techniques in each of the three dose groups. $K\beta_1$[Pb] consistently overestimated AAS[Pb], but the difference between predicted $K\beta_1$[Pb] and AAS[Pb] decreased as the dose increased. For the 75 µg/kg/day dose group, the difference between predicted AAS[Pb] and $K\beta_1$[Pb] was –7.1 µg/g (SE 1.2 µg/g; p < 0.0001; 95% CL, –9.44 to –4.67 µg/g); for the 225 µg/kg/day dose group, the difference was –6.11 µg/g (SE 0.90 µg/g; p < 0.0001; 95% CL, –7.93 to –4.28 µg/g); and for the 675 µg/kg/day dose group, the difference was –3.26 µg/g (SE 1.40 µg/g; p = 0.0252; 95% CL, –6.09 to –0.43 µg/g). The difference between predicted AAS[Pb] and $K\beta_1$[Pb] at the mean dose of 330.7 µg/kg/day was –5.4 µg/g (SE 0.84 µg/g; p < 0.0001; 95% CL, –7.12 to –3.75 µg/g).

The situation for XRF[Pb] (the single estimate of lead concentration obtained by combining $K\alpha_1$[Pb] and $K\beta_1$[Pb]) was intermediate between those for $K\alpha_1$[Pb] and $K\beta_1$[Pb], as expected. When comparing XRF[Pb] to AAS[Pb], mixed modeling (for which there were no influential observations) showed that both dose and technique were significant factors and that technique × dose was of borderline significance (p = 0.08). Again, the marginal significance of technique × dose indicates that the relationships between lead concentration and dose differed for the two techniques: The lines had slopes and intercepts that were significantly different. The relation for AAS[Pb] is given above. The relation for XRF[Pb] was:

XRF[Pb] = 4.8116 (± 0.9778; p < 0.0001)
+ [0.0162 (± 0.0023; p < 0.0001) × dose].

There was a statistically significant difference between the lead concentrations obtained by the two techniques in both the 75 µg/kg/day and 225 µg/kg/day dose groups, but no significant difference in the 675 µg/kg/day dose group. XRF[Pb] consistently overestimated AAS[Pb], but the difference between the two techniques decreased as dose increased. For the 75 µg/kg/day dose group, the difference between predicted AAS[Pb] and XRF[Pb] was –3.86 µg/g (SE 1.02 µg/g; p = 0.0005; 95% CL, –5.92 to –1.79 µg/g); for the 225 µg/kg/day dose group, the difference was –3.10 µg/g (SE 0.78 µg/g; p = 0.0003; 95% CL, –4.68 to –1.52 µg/g); and for the 675 µg/kg/day dose group, the difference was –0.81 µg/g (SE 1.21 µg/g; p = 0.5070; 95% CL, –3.26 to –1.64 µg/g). The difference between predicted AAS[Pb] and XRF[Pb] at the mean dose of 330.7 µg/kg/day was –2.56 µg/g (SE 0.72 µg/g; p = 0.0010; 95% CL, –4.02 to –1.10 µg/g).

The results of regression analysis and the correlation coefficients, performed to allow comparison to the published literature, are shown in Table 3. Paired t-tests showed *a*) no significant difference between predicted $K\alpha_1$[Pb] and AAS[Pb] in any of the three lead dose groups or in the data from all three lead dose groups combined; *b*) a significant difference between predicted $K\beta_1$[Pb] and AAS[Pb] in all three lead dose groups (although the difference in the 675 µg/kg/day dose group was of borderline significance; p = 0.0465) and in the data from all three lead dose groups combined; and *c*) a significant difference between predicted XRF[Pb] and AAS[Pb] in the 75 µg/kg/day and 225 µg/kg/day dose groups; no significant difference in the 675 µg/kg/day dose group; and a significant difference in the data from all three lead dose groups combined.

Discussion

In our analysis of agreement, AAS is considered to be the reference method, but agreement between XRF and AAS does not preclude, of course, the presence of a systematic bias in each of the techniques.

Qualitatively, the agreement between $K\alpha_1$[Pb] and AAS[Pb] appears to be good, but the agreement between $K\beta_1$[Pb] and AAS[Pb] is not. There are several possible reasons for this. For example, Somervaille et al. (*11*), in measurements of human tibiae, found no evidence that random differences between the two techniques were greater than could be accounted for by the measurement variance of each technique; but they noted that any inhomogeneity in lead concentration may contribute unaccounted-for random error. Such inhomogeneity may also account partly for the differences between XRF and AAS in our measurements. So, too, may the fact that AAS was performed on the right femur whereas XRF was performed on the left femur. However, we do not expect this difference to be important, because a previous study (*4*) has shown no difference between measurements of left and right human tibiae with the limits of precision of those authors' AAS method.

Table 3. Ordinary least squares (unweighted) regression and correlation coefficients between XRF-measured and AAS-measured lead concentration in 44 swine femurs.

Dependent variable	Independent variable	Intercept (± SE) (p-Value)	Slope (± SE) (p-Value)	p-Value for testing H_0:slope = 1	Pearson r	Spearman rank r (p-Value)
$K\alpha_1$[Pb]	AAS[Pb]	1.8 (± 1.4) (0.2)	0.71 (± 0.14) (< 0.0001)	0.0474	0.6149	0.5815 (< 0.0001)
$K\beta_1$[Pb]	AAS[Pb]	8.3 (± 1.2) (< 0.001)	0.62 (± 0.13) (< 0.0001)	0.0042	0.6028	0.5855 (< 0.0001)
XRF[Pb]	AAS[Pb]	5.1 (± 1.1) (< 0.001)	0.67 (± 0.11) (< 0.0001)	0.0043	0.6904	0.6612 (< 0.0001)

We believe it most likely that the reason for the discrepancy in $K\beta_1$[Pb] is technical rather than biologic: namely, the X-ray peak area extraction program parameter for a calcium/phosphorous-related spectrum feature (the amplitude, as a fraction of the coherent scatter peak, of the discontinuity in the distribution of photon counts arising from Compton scattering off tightly bound electrons from calcium and phosphorous). It is possible that the value used for this fitting parameter is not optimal for measurements of swine femur. The parameter is treated as a constant, although it is calculated using assumed compositions of human bone. Any difference between the composition of human and swine bone would affect the calculated value of this parameter. There were too few data to show a clear minimum in the sum of the squares of the differences between $K\beta_1$[Pb] and AAS[Pb] when we manually adjusted the fitting parameter; we therefore had insufficient grounds for determining whether an incorrect value for this parameter was partially or wholly responsible for the bias between $K\beta_1$[Pb] and AAS[Pb]. To use XRF as a substitute for AAS measurements of swine femur, only $K\alpha_1$[Pb] would be used until the bias between $K\beta_1$[Pb] and AAS[Pb] is explained and corrected.

The SD of repeated XRF measurements of the swine femur was greater than that obtained from repeated measurements of human bones (data not shown), a result of the smaller size of the swine femurs. Our quantitative estimates of the confidence limits for the agreement between XRF and AAS probably overestimate (i.e., are wider than) the confidence limits that would be obtained from a comparison between XRF and AAS performed on human bones. Nevertheless, we can compare our data to those of previous reports.

One previous study compared a substantial number (80) of XRF and AAS measurements from 30 human bone samples that underwent both AAS and XRF analysis (*11*). In that study, Somervaille et al. measured human bone samples (mainly tibiae) and calculated the mean difference and the SD of the differences. From these, they calculated the *t* statistic and the probability (90%) that there was no difference between the two sets of measurements. Not all the data used to calculate these statistics were from independent samples, and we therefore recalculated them from the data presented by Somervaille et al. (*11*): XRF[Pb] underestimated AAS[Pb] by 0.63 μg/g, and the SD of the differences was 6.79 μg/g. The mean difference in our study (XRF[Pb] overestimated AAS[Pb] by 2.6 μg/g) was significantly (p = 0.0218) worse than that reported by Somervaille et al. (*11*). The SD of the differences in our study (4.9 μg/g) was less than that calculated from the data of Somervaille et al. (*11*), but the difference was of borderline significance (p = 0.044).

Somervaille et al. (*11*) also calculated χ^2 and found that the uncertainties associated with XRF and AAS were consistent with the SD of the differences between the two techniques. This calculation requires, for several stages of the AAS measurement process, knowledge of the measurement uncertainty that was unavailable to us. However, Somervaille et al. (*11*) assigned a ± 10% uncertainty to all the AAS data. We therefore calculated χ^2 for our data for three AAS measurement uncertainty levels: ± 10% per Somervaille et al. (*11*); ± 1 μg/g (the detection limit); and ± 1/2 μg/g (half the detection limit). All χ^2 tests were failed (regardless of the level of uncertainty assigned to the AAS data and regardless of whether the entire data set or an individual concentration group was considered), indicating that the SD of our differences between XRF[Pb] and AAS[Pb] was not consistent with the uncertainties assigned to each technique. For completeness, we note that Somervaille et al. (*11*) found no significant linear trend in their differences between XRF[Pb] and AAS[Pb], and we found no nonlinear trend.

Hu et al. (*12*) performed XRF and AAS at eight locations on three human legs. The agreement between XRF and AAS was given as a correlation coefficient (0.98), a slope (1.02, uncertainty not given), and an intercept (–1 μg/g, uncertainty not given). Our swine femur data showed a lower Pearson correlation coefficient, a slope that was significantly different from unity (suggesting a proportional bias), and an intercept that was significantly different from zero (suggesting a fixed bias).

Aro et al. (*13*) measured tibia and patella lead concentrations via both ^{109}Cd-based KXRF and inductively coupled plasma mass spectrometry (ICPMS) in eight amputated cadaver legs. The authors quote only correlation coefficients but present their data allowing the mean and standard deviation of the differences between XRF and ICPMS to be calculated along with the limits of agreement. The data of Aro et al. (*13*) yield a mean (± SD) difference of –0.7 (± 2.7) μg/g and –1.2 (± 1.8) μg/g for intact tibiae and patellae respectively. The sign of each mean difference indicates that XRF underestimated the bone lead concentration obtained by ICPMS. The mean difference between XRF[Pb] and AAS[Pb] in the swine femur was not quite significantly worse (greater) than the mean difference between the XRF and ICPMS tibia measurements of Aro et al. (*13*) (p = 0.076). There was no more variability in the differences between XRF[Pb] and AAS[Pb] in our study of swine femurs than in the human tibia data of Aro et al. (*13*), but the difference was also of borderline significance (p = 0.0997).

Conclusion

The agreement (i.e., the mean difference) between XRF and AAS measurement of swine femur lead concentrations is similar to but worse than the agreements reported between XRF and AAS measurements of human tibiae. Swine bones may require adjustment of the XRF spectrum $K\beta_1$ peak extraction method. The findings of this study are applicable only to measurements of swine femurs using our method for ^{109}Cd-based KXRF and may not necessarily extend to the technique in general.

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