Evaluation of a low-cost commercially available extraction device for assessing lead bioaccessibility in contaminated soils

Clay M. Nelson*[a], Thomas M. Gilmore,[b] James M. Harrington,[c] Kirk G. Scheckel,[d] Bradley W. Miller[c] and Karen D. Bradham[a]

[a] *U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, Research Triangle Park, North Carolina 27711*
[b] *National Caucus and Center on Black Aged, Inc., Senior Environmental Employment Program, Durham, North Carolina 27713*
[c] *Oak Ridge Institute for Science and Education, Oak Ridge, Tenessee 37831*
[d] *U.S. Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Cincinnati, Ohio 45224*

** **Corresponding author: Clay Nelson** Postal Address: 109 T.W. Alexander Drive, Mail Code D205-05, Durham, NC 27711, phone: (919) 541-5617, fax; (919) 541-3527 email: nelson.clay@epa.gov*

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Abstract

The U.S. EPA's *in vitro* bioaccessibility (IVBA) method 9200.1-86 defines a validated analytical procedure for the determination of lead bioaccessibility in contaminated soils. The method requires the use of a custom-fabricated extraction device that uses a heated water bath for sample incubation. In an effort to improve ease of use, increase sample throughput, and reduce equipment acquisition and maintenance costs, an alternative low-cost, commercially available extraction device capable of sample incubation via heated air and end-over-end rotation was evaluated. An intra-laboratory study was conducted to compare lead bioaccessibility values derived using the two extraction devices. IVBA values were not statistically different ($\alpha = 0.05$) between the two extraction devices for any of the soils (n=6) evaluated in this study, with an average difference in mean lead IVBA of 0.8% (s.d. = 0.5%). The commercially available extraction device was able to generate accurate lead IVBA data as compared to the U.S. EPA's expected value for a National Institute of Standards and Technology standard reference material soil. The relative percent differences between high and low IVBA values for each soil, a measure of instrument precision, were also not statistically different ($\alpha = 0.05$) between the two extraction devices. The statistical agreement of lead IVBA values observed using the two extraction devices supports the use of a low-cost, commercially available extraction device as a reliable alternative to a custom-fabricated device as required by EPA method 9200.1-86.

Keywords: human exposure, lead, bioavailability, bioaccessibility, soil, risk assessment

Environmental Impact

In 2008, the U.S. EPA developed a standard operating procedure (EPA method 9200.1-86) for measuring lead bioaccessibility in soil used by many research labs throughout the U.S and internationally. This work supports the refinement of EPA method 9200.1-86 to allow for the use of a commercially available extraction device as an alternative to the custom fabricated device previously required by the method, reducing equipment acquisition and maintenance costs, improving instrument ease of use, and increasing sample throughput. It is also hypothesized that use of a commercially available device will improve precision in lead bioaccessibility results across multiple laboratories tasked with assessing human health risks from ingestion of lead-contaminated soils.

Introduction

Lead is the most common inorganic contaminant at Superfund sites. [1] Exposure to lead can result in acute and chronic toxicity, including adverse health impacts to the nervous system, kidney damage, and anemia. [2] Lead exposure is a particular public health concern for children, and increasing scientific evidence shows that even low levels of lead exposure can cause deleterious and irreversible health effects. [3-7] In response, the U.S. Centers for Disease Control and Prevention (CDC) recently revised their lead poisoning guidelines for children, reducing blood lead levels of concern from 10 µg/dL to 5 µg/dL. [8] Ingestion of lead-contaminated soils often drives risk assessments for human exposure to metal contaminants at Superfund sites. [9] With increasing urbanization, exposure to lead-contaminated soils grows more likely as residential areas extend into the vicinity of or, in some cases, intrude onto Superfund sites. [10]

Reliable analysis of human health risks from ingestion of lead-contaminated soils depends on estimating the bioavailability of lead in soil, [11] defined by the U.S. EPA as the fraction of an ingested dose that crosses the gastrointestinal epithelium and becomes available for distribution to internal target tissues and organs. [12] Current exposure estimates from ingestion of lead-contaminated soils often do not consider differences between the bioavailability of metals in water and soil. [13, 14] The use of default values that assume equivalent bioavailabilities for metals in the two matrices can affect the accuracy in predicted risk associated with ingestion of lead-contaminated soil. [15] Animal models have been developed to estimate soil-specific lead bioavailability; [16-19] however, time and cost considerations often limit their use in risk assessment. [20] Efforts have been made to develop accurate and inexpensive *in vitro* extraction tests that are predictive of *in vivo* bioavailability. [20-22] These methods are based on the concept that lead solubilization in gastric fluid (referred to as bioaccessibility) is an important determinant of lead bioavailability. [14]

An *in vitro* assay for the determination of lead bioaccessibility in contaminated soils has been validated by the U.S. EPA against *in vivo* bioavailability data. [14] This method resulted in standard operating procedure (SOP) 9200.1-86 produced by the U.S. EPA. The SOP requires the use of a custom-fabricated extraction device that uses an electric motor to operate a block that holds 125-mL high-density polyethylene bottles rotated end over end at 30±2 revolutions per minute (RPM) inside a water bath heated by an immersion circulating heater at 37±2 ^{o}C. [23]

The use of a custom-fabricated extraction device presents challenges that may impact the ability of this method to be successfully employed by the many research laboratories tasked with assessing human health risk from lead contaminated sites. Such challenges include higher equipment costs for custom-fabrication and access to the technical expertise necessary to build and maintain the

equipment. Potential impacts on accuracy and precision of IVBA estimates across multiple labs may also result from variations in equipment design and fabrication from laboratory to laboratory.

The U.S. EPA recently completed a round robin study to develop consensus lead bioaccessibility values for National Institute of Science and Technology (NIST) Standard Reference Materials (SRMs) 2710a and 2711a, and revised EPA method 9200.1-86 to incorporate these new consensus values. [24] Two of the eight labs that participated in the round robin study used extraction devices that heated samples via air incubation. Preliminary research by our laboratory evaluated whether sample heating via air versus water incubation affected observed mean lead IVBA values. There was no statistically significant difference in lead IVBA values between the heating methods for NIST SRM 2710a. [24] Based on this preliminary research, we investigated whether a low-cost commercially-available extraction device(s) that uses heated air for sample incubation could be used as a reliable alternative to a custom-fabricated device for the determination of lead IVBA across multiple soils.

Materials and Methods

In this study, an intra-laboratory comparison was performed between lead IVBA data derived using a custom-fabricated extraction device similar to that described in EPA method 9200.1-86 and an alternative low-cost, commercially-available extraction device that uses heated air for sample incubation (Figure 1).

Soil origin, processing, and physicochemical characterization

Six soils were included in this study. Two soils were collected from sites impacted by mining and smelter activities and three soils were collected from sites impacted by agricultural pesticide applications. Soils were dried (< 40 °C) and sieved to < 250 μm, the particle size fraction considered by the U.S. EPA to be representative of that which adheres to hands and be subsequently ingested by hand-to-mouth contact, especially in young children. [9, 14] Soil samples were homogenized, riffled, and aliquots were split for each extraction method following procedures described in Blume et al. [25] A NIST SRM (Montana soil 2710a) was also included in this study and used as purchased from NIST. [26] Total soil Pb content was determined by extracting the < 250 μm soil in accordance with U.S. EPA Method 3051A [27] with analysis by Varian Vista-MPX Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) according to U.S. EPA Method 6010C. [28]

***In vitro* bioaccessibility assays**

Pb bioaccessibility values were derived in accordance with EPA method 9200.1-86, which has been validated by the U.S. EPA against *in vivo* relative bioaccessibility data, using two extraction devices (Figure 1). The first device was a custom-fabricated plexiglass water tank, similar to that described in the standard operating procedure for EPA method 9200.1-86, fitted with a rotary sample holder capable of holding up to twelve 125-mL high density polyethylene sample bottles with end-over-end mixing and sample heating via a circulating water pump. IVBA results derived using this device were compared to those using a commercially-available hybridization oven (Amerex Instruments, Inc. model HS-101) capable of holding sixteen 125-mL HDPE sample bottles and rotating samples end-over-end via a rotating carousel. Oven temperature was controlled via a circulating air pump.

For both extraction devices, one gram of test soil was added to 100 mL of buffered glycine solution pre-heated to 37±2 °C in a clean 125-mL HDPE bottle. A 0.4 M glycine solution was prepared by adding glycine (Sigma Aldrich BioXtra) in deionized water and acidifying to pH 1.5 by addition of concentrated hydrochloric acid (SCP Science PlasmaPure). Samples were rotated end-over-end at 30±2 RPM and 37±2 °C for one hour. Other rotation speeds ranging between 10 and 30 RPM were also investigated in this study to evaluate possible impacts of rotational speed on lead IVBA values. Three replicates of each test soil were extracted per batch. This was repeated four times per soil for each extraction device for a total of 12 samples per test soil per extraction device. Blanks, a NIST SRM 2710A soil and spikes were also analyzed with each batch to meet quality assurance and quality control (QA/QC) requirements defined in EPA method 9200.1-86. All labware were cleaned, acid washed and triple rinsed with deionized water prior to use. Immediately following the one-hour incubation, extraction solutions were filtered using a 0.45 µm filter (Whatman GD/X) and refrigerated at 4 °C until subsequent analysis. Extractable Pb was determined by ICP-MS in accordance with U.S. EPA method 6020A. [29] Analysis of the *in vitro* extracts included QA/QC procedures as described in EPA Method 6020A. The method detection limit for Pb in extraction fluid was 0.02 µg L^{-1}.

The *in vitro* bioaccessibility (IVBA) of lead from each soil was calculated using the following equation:

$$IVBA\ (\%) = \frac{in\ vitro\ extractable\ Pb(mg\ kg^{-1})}{total\ Pb\ in\ soil\ (mg\ kg^{-1})}\ x\ 100$$

Statistical Analysis

Differences in mean Pb bioaccessibility and relative percent difference (RPD) derived using the two extraction devices were compared by t-test (R version 2.9.1). To evaluate precision of the two extraction devices, relative percent difference (RPD) in the highest and lowest observed IVBA values (n = 12) observed for each soil were compared. Precision was also evaluated qualitatively by overlaying Gaussian probability density curves on histograms of observed IVBA results using the HISTGAUS.XFM transform in SigmaPlot (Version 11.0). To evaluate accuracy, RPD in IVBA values for NIST 2710a were compared to a consensus value derived from an inter-laboratory round robin study. [24]

Results and Discussion

In vitro bioaccessibility results

Total soil Pb concentrations of the test soils ranged from 420 to 5100 ppm (Table 1). Mean observed lead IVBA values ranged from 21.6 to 84.5 %. IVBA values for a particular test soil were not statistically different (α =0.05) between extraction devices for any of the six soils tested in this study, with an average difference in Pb IVBA means of 0.8% (s.d. = 0.5%). A preliminary investigation into the impacts of varying instrument rotational speed between 10 and 30 RPM did not suggest that rotational speed within this range was an important factor in observed lead IVBA values (data not shown); however, one should adhere to the recommended rotational speed of 30±2 RPM as outlined in EPA method 9200.1-86 when conducting these studies.

Quality control data are summarized in Table 2. All observed QC data were within acceptable control limits, as defined by EPA method 9200.1-86, with the exception of matrix spike recovery values for Soil 5. Lead values in blank samples containing only buffered glycine solution were at or below 11.5 ppb using either extraction device. Buffered glycine solutions (blanks) spiked with 2 ppm soluble lead averaged 96.9 and 96.1% recovery in the commercially available hybridization oven and custom fabricated extraction device respectively. Matrix spike recoveries (test soils in buffered glycine solution spiked with 2 ppm soluble lead) averaged across all six test soils were 93.2 and 94.3% using the commercially available hybridization oven and custom fabricated extraction device respectively. Matrix spike recovery values for soil 5, however, were below the minimum acceptable threshold of 75 % recovery using both extraction devices, with an average recovery of 56.0 % (range of 52.9 to 60.4 %, n = 4) and 54.6 % (range of 52.3 to 56.9 %, n = 4) using the commercially available hybridization oven and custom fabricated extraction devices, respectively. Removing soil 5 resulted in improved matrix spike recovery values of 100.7 and

102.2%. While Soil 5 matrix spike recovery values did not fall within acceptable QC limits defined by EPA method 9200.1-86, of importance for this study was that both extraction devices performed similarly with respect to observed matrix spike recovery values. An investigation into the possible mechanism of reduced matrix spike recovery for Soil 5 was not part of this study. However, one possible explanation is that the physicochemical properties of this particular soil (i.e., high concentration of iron oxides) caused the adsorption of soluble Pb onto the soil matrix, converting it into a non-bioaccessible form.

The ability of an extraction device to reliably generate IVBA values within 10% RPD of an expected value using a NIST SRM soil, a measure of instrument accuracy and precision, is a requirement of EPA method 9200.1-86. An inter-laboratory study conducted by the U.S. EPA reported a mean Pb IVBA value for NIST 2710a of 67.5%. [24] The mean Pb IVBA value (n=12) for NIST 2710a observed in this study using the commercially available hybridization oven was 65.4% with a mean RPD of 3.2% (range of 0.6 to 7.5 %) from the round-robin expected value. The observed mean IVBA value for NIST 2710a using the custom fabricated extraction device was 64.1%, with a mean RPD of 4.6% (range of 0.1 to 7.7 %) from the round-robin expected value. All observed RPD values were within the ±10% RPD control limit as required by EPA method 9200.1-86.

An extraction device must also be capable of generating precise lead IVBA data across multiple sample replicates. EPA method 9200.1-86 sets a control limit of ±20% RPD between sample replicates. Table 1 shows RPD values between the highest and lowest observed Pb IVBA values for each of the test soils using each extraction device (n = 12). Observed values ranged from 7.8 to 17.5 % (mean of 11.3%) and 5.1 to 15.6 % (mean of 9.2%) for the commercially available hybridization oven and custom fabricated extraction device, respectively. All observed RPD values were below the 20% threshold for acceptance with EPA method 9200.1-86. RPD values derived from the two extraction devices were not statistically different at $\alpha = 0.05$ ($P = 0.28$; two-tailed, paired t-test), indicating that variation in precision in IVBA values across sample replicates for a particular soil were consistent using both extraction devices.

In addition to comparing RPD in extreme IVBA values, overall distributions of observed IVBA values using the two extraction devices were evaluated qualitatively by overlaying Gaussian probability density curves on histograms of IVBA results for each test soil (Figure 2). To facilitate comparisons between soils, the x-axis scale has been normalized to represent ±20% RPD values about the combined mean IVBA. A qualitative comparison showed similar distributions in observed IVBA values between the two extraction devices. Observed variations in Pb IVBA precision across

multiple soils investigated in this study may be due to characteristics of the soils or variations in how each soil was collected in the field and processed for analysis.

Conclusion

This study compared intra-laboratory differences in Pb IVBA values across multiple soils derived using two extraction devices that differed primarily in sample heating method (i.e., water versus air incubation). The lack of statistically significant differences in mean IVBA values for any of the six soils investigated and similar performance with respect to accuracy and precision in IVBA values suggests that a low-cost, commercially available extraction device capable of rotating samples end-over-end at 30±2 RPM and incubating samples via heated air at 37±2 °C can reliably generate Pb IVBA values similar to those derived using a custom-fabricated device as described in EPA method 9200.1-86. Observed QA/QC data also suggest that the commercially-available device can produce QC data of similar quality to those derived using a custom-fabricated device.

The potential for lower acquisition and maintenance costs, ease of use, and higher sample throughput capabilities of a commercially-available extraction device provides an attractive, reliable, and inexpensive alternative to that of an independently customized and fabricated device, as is currently used by many labs conducting IVBA research in accordance with EPA method 9200.1-86. While this study compared intra-laboratory differences between extraction devices, an inter-laboratory analysis may be helpful to further investigate accuracy and precision in IVBA values using different extraction devices across multiple laboratories, as variations in parts and equipment used in custom fabrication may impact the accuracy and precision in IVBA results. The use of similar equipment may provide more consistent IVBA results across multiple laboratories for use in human health risk assessments. With the growing body of studies suggesting that even low levels of lead exposure can be harmful, particularly to children, improvements in tools and methods to reliably and accurately measure the bioaccessibility of lead in contaminated soils are increasingly important.

Table 1 Comparison of Pb bioaccessibility results derived using the two extraction devices.

soil ID	soil type	soil [Pb] (mg/kg)	commercially available hybridization oven		custom fabricated extraction device		P-value[c]
			IVBA (%)[a]	RPD[b]	IVBA (%)	RPD	
1	Orchard	1360	75.6	13.6	74.8	5.1	0.39
2	Orchard	2070	84.5	10.2	83.4	13.6	0.44
3	Orchard	1660	68.3	10.3	68.7	6.1	0.59
4	Mining/Smelter	3500	21.6	8.4	21.8	5.5	0.41
5	Mining/Smelter	420	57.3	17.5	56.3	15.6	0.42
2710A	SRM	5100	65.4	7.8	64.1	9.2	0.11

[a] Mean of 12 replicates. [b] Relative percent difference between lowest and highest observed values (n=12). [c] From t-test comparing mean IVBA values between the two extraction devices for each soil

Table 2 Results of quality control data during extraction test.

analysis	frequency[a]	control limit[b]	observed values	
			commercially available hybridization oven	custom fabricated extraction device
reagent blank	once/batch	< 25 µg/L	1.0 to 11.5 µg/L (mean = 2.6, n = 8)	0.8 to 5.9 µg/L (mean = 1.9, n = 12)
bottle blank	once/batch	< 50 µg/L	0.9 to 2.4 µg/L (mean = 1.3, n = 8)	0.8 to 6.3 µg/L (mean = 1.9, n = 12)
NIST 2710a SRM	once/batch	± 10% RPD	0.6 to 7.5% (mean = 3.2%, n = 8)	0.1 to 7.7% (mean = 4.7%, n = 12)
blank spike (10 mg/L)	once/batch	85-115% recovery	94.0 to 110.0% (mean = 96.9%, n = 8)	91.4 to 110.7% (mean = 96.1%, n = 12)
matrix spike (10 mg/L)	once/soil/batch	75-125% recovery	52.9 to 108.2% (mean = 93.4%, n = 24)	52.3 to 117.7% (mean = 94.3%, n = 24)
duplicate sample	multiple/soil/batch	± 20% RPD[c]	7.8 to 17.5% (mean = 11.3, n = 6)	5.1 to 15.6% (mean = 9.2, n = 6)

[a] Frequency of QC data for this study met or exceeded guidelines established in EPA method 9200.1-86. [b] As set by EPA method 9200.1-86. [c] Reported RPD values are based on high and low IVBA values observed for each soil (n=12).

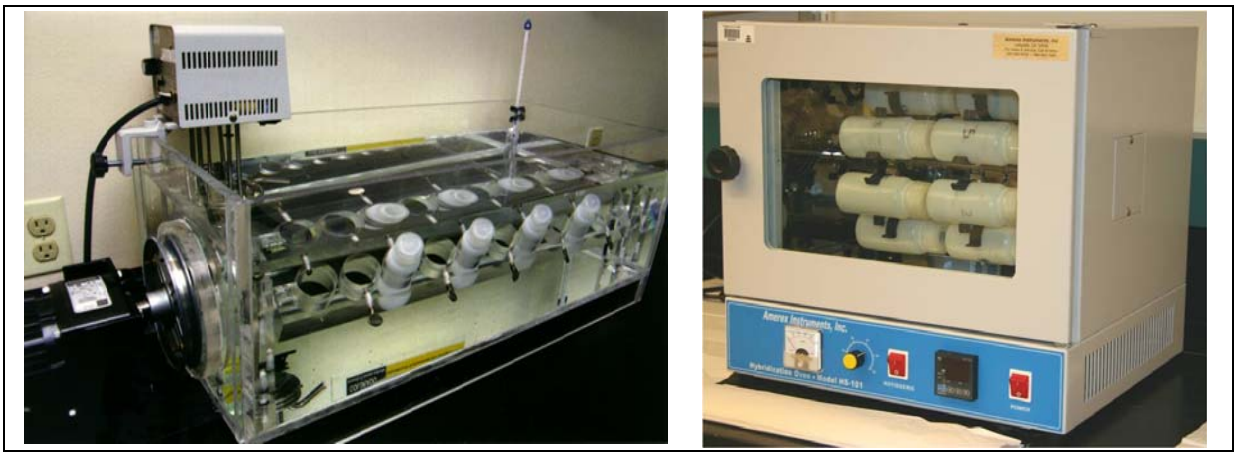

Fig. 1 Images of custom-fabricated extraction device (left) and commercially-available hybridization oven (right) evaluated in this study.

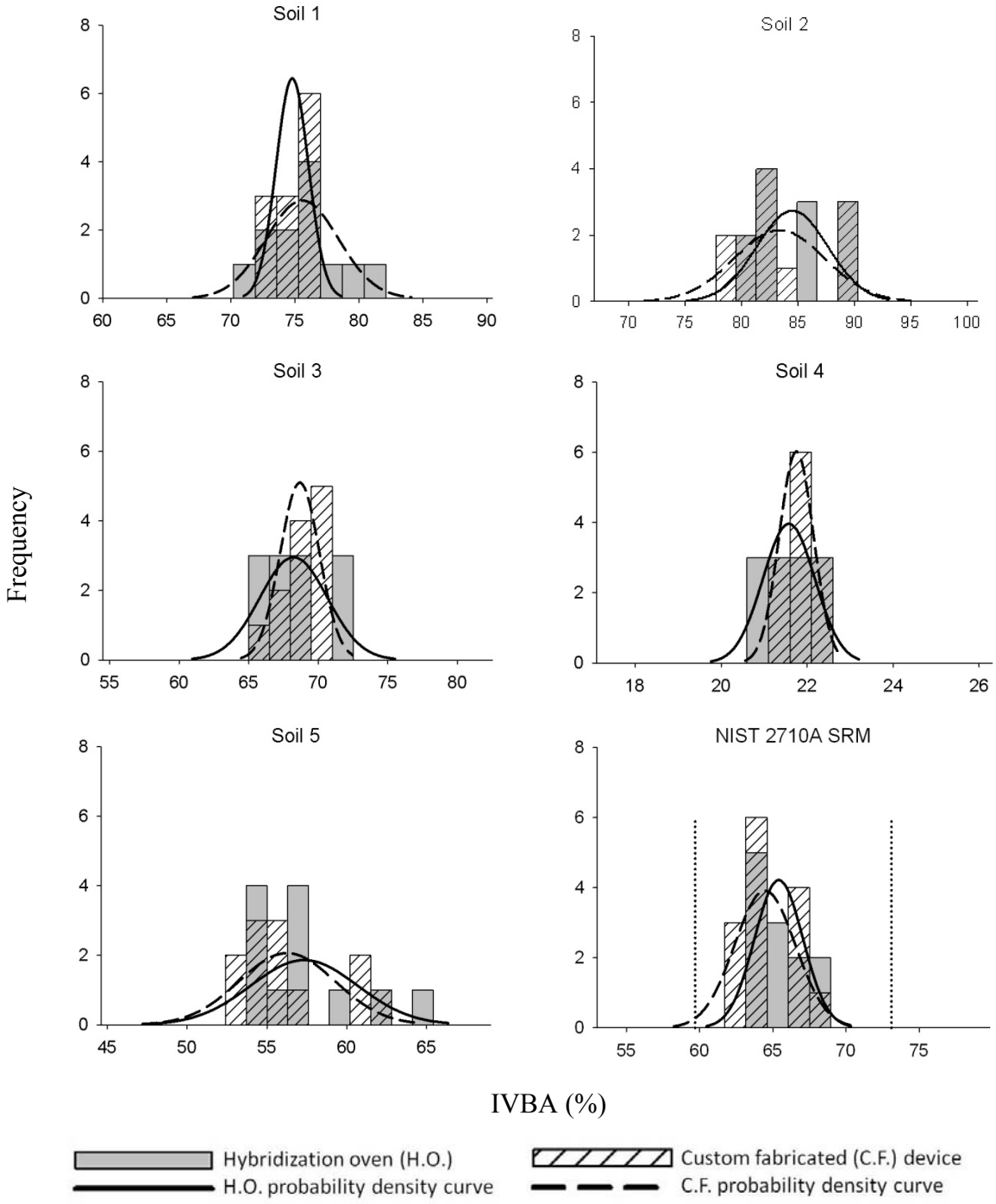

Fig. 2 Gaussian probability density curves overlaid on histograms of observed IVBA values (12 replicates per soil per extraction method). The vertical dotted lines for the NIST SRM soil represents the ±10% RPD values about an expected value of 67.5% derived from an inter-laboratory round robin study. To facilitate comparisons between soils, the x-axis scale has been normalized to represent ±20% RPD values about the combined mean IVBA value.

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