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Does EPA Method 7471 Accurately Measure Mercury Concentrations in Fish?

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Introduction and Background

Primarily for the purpose of monitoring for mercury levels in fish and in support of consumption advisories, many environmental laboratories routinely utilize a common testing method, EPA Method 7471A: *Mercury in Solid or Semisolid Waste* (EPA 7471), for the preparation and analyses of biota samples for total mercury concentrations.

When the results of recent analyses for methylmercury concentrations in a set of fish tissue samples (through a modification of EPA Method 1630: *Methylmercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*) exceeded the corresponding results for total mercury by EPA 7471 (performed by a different laboratory), concerns were raised about the accuracy of the data.

Figure 1: rainbow trout (*Oncorhynchus mykiss*)

This same set of fish tissue samples was then analyzed by EPA Method 1631, Revision E: *Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry* using an approved modification developed for the preparation of solid samples entitled *Appendix to Method 1631: Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation* (EPA 1631) to provide confirmation of the total mercury concentrations.

The results achieved using EPA 1631 were consistently higher than the results reported when using EPA 7471 and corresponded more appropriately with the results of the methylmercury analyses, suggesting that the EPA 7471 results were possibly biased low.

An investigation was performed to explore the relative accuracy of each method and the potential sources of any discrepancies.

Experiments

Tissue samples were collected from 21 freshwater fish of various species and were prepared and analyzed by methods EPA 7471 and EPA 1631 concurrently.

Quality control samples for each method included the analyses of three of the fish samples in duplicate to demonstrate method precision, four matrix spikes of the fish samples to identify any potential matrix-related interferences, and a standard reference material in duplicate, IAEA-407 *Fish Homogenate* (International Atomic Energy Agency, Vienna, Austria), to demonstrate the efficiency of the digestion methods and overall method accuracy.

Materials & Equipment

Samples were prepared for EPA 7471 using concentrated nitric acid (HNO_3), concentrated hydrochloric acid (HCl), (Fisher Scientific, Fair Lawn, New Jersey), and hydroxylamine hydrochloride with potassium permanganate ($KMnO_4$) (J.T. Baker Chemical Company, Phillipsburg, New Jersey) as the oxidizing reagent.

The samples for EPA 7471 were prepared in 50 mL polypropylene snap-top digestion vessels and heated in an Environmental Express Hot Block (Environmental Express, Charleston, South Carolina).

Samples were prepared for EPA 1631 using HNO_3, concentrated sulfuric acid (H_2SO_4) (Fisher Scientific), and hydroxylamine hydrochloride with bromine monochloride (BrCl) as the oxidizing reagent.

The samples for EPA 1631 were prepared in 40 ml glass vials (Fisher Scientific) and heated on a hotplate.

All prepared samples were then analyzed by cold vapor stannous chloride ($SnCl_2$) reduction, followed by gold amalgamation, thermal desorption, and atomic fluorescence spectroscopy (CVAFS) using a Brooks Rand Labs MERX® Total Mercury Analyzer.

It should be noted that the polypropylene digestion vessels, as opposed to the glass vials used in EPA 1631, are called for in EPA 7471 and were also necessary due to the required volume of reagents.

Volatile-mercury loss from the polypropylene vials does not appear to be an issue if the samples are analyzed immediately subsequent to reduction; however, later reanalyses (3 days) have shown a significant loss of volatile-mercury and the samples must be reprepared if reanalyses are required.

Figure 2: MERX-1400

Comparison of Digestion Procedures

All of the 21 fish tissue samples and associated quality control samples were prepared according to EPA 7471 and EPA 1631 using the materials and equipment described before.

Summaries of the heating time, digestion reagents, and oxidation reagents are described in Table 1.

It was plainly observed that even after the digestion procedure according to EPA 7471 was completed, visible particulate matter remained settled at the bottom of the digestion vessels.

Figure 3 shows a rack of samples digested according to EPA 7471 and the remaining visible particulate matter due to incomplete dissolution of the fish tissue.

Figure 4 shows a comparison of two samples digested according to EPA 1631 (outside glass vials) with two samples digested according to EPA 7471 (inside polypropylene vials).

As can be seen in the figures, the samples digested according to EPA 1631 do not contain any visible particulate matter in contrast to those digested according to EPA 7471.

This is potentially due to the types and volumes of digestion reagents used, as well as the longer heating time.

Parameter	EPA 1631	EPA 7471
Heating Time	4 hours	30 minutes
Digestion Reagents	$HNO_3 + H_2SO_4$	HNO_3 + HCl
Oxidation Reagents	BrCl	$KMnO_4$

Table 1: Digestion Procedures

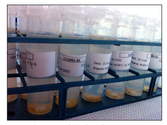

Figure 3: incomplete dissolution

Figure 4: comparison of digestates

Sample Results

The prepared samples were then analyzed according to either EPA 7471 or EPA 1631, according to the digestion procedure employed.

Figure 5 shows the results for all 21 fish tissue samples analyzed by both EPA 7471 and EPA 1631.

Just as was observed in the earlier comparison that initiated this investigation, the results of the analyses by EPA 1631 are consistently greater than the results of the analyses by 7471 (an average of 20%).

The conventional technique for determining which of these sets of results is more accurate is to evaluate the precision and accuracy of the methods as demonstrated through the analyses of various types of quality control samples.

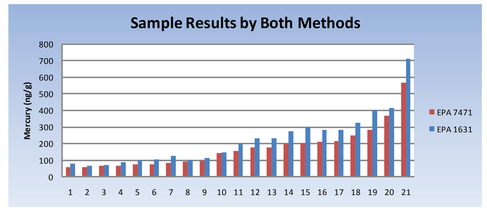

Figure 5

Quality Control Analyses

The three samples that were selected to be analyzed in duplicate by both EPA 7471 and EPA 1631 (identical samples by both methods) predictably produced results that were lower by EPA 7471 (Figure 6) than by EPA 1631 (Figure 7), but the relative percent difference (RPD) of the results of all six of the duplicate analyses were well within the most stringent criteria for precision.

With an average RPD of approximately 4% by both methods, it can be safely assumed that each is capable of producing highly reproducible results.

However, when the four matrix spikes (MS) of the fish tissue samples (two pairs of identical samples by both methods) were analyzed (Figure 8), the discrepancies between the expected recoveries were significant and consistent.

The average recoveries for the MSs analyzed by EPA 7471 were only 77%, while the average recoveries for the MSs analyzed by EPA 1631 were 102%.

The differences in these recoveries are consistent with the discrepancies observed with the results from the analyses of the normal fish tissue samples.

Moreover, this same low-bias was also observed in the results of the duplicate analyses of the standard reference material (SRM) IAEA-407 (Figure 9).

The results of these quality control analyses indicate that while the results of the analyses performed by EPA 7471 are relatively precise, there is a consistently low-bias of approximately 20% relative to expected values and those achieved by using EPA 1631.

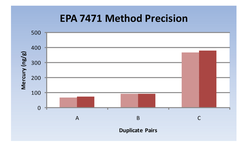

Figure 6

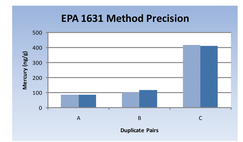

Figure 7

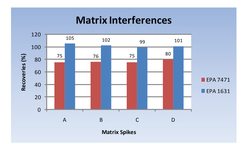

Figure 8

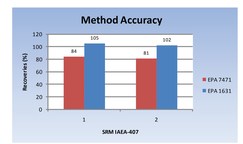

Figure 9

Conclusion

Method 7471 is approved specifically only for "soils, sediments, bottom deposits, and sludge-type materials." Moreover, the method also clearly states:

> *"All samples must be subjected to an appropriate dissolution step prior to analysis. If this dissolution procedure is not sufficient to dissolve a specific matrix type or sample, then this method is not applicable for that matrix."*

Through comparison of the results achieved by EPA 7471 with those achieved by EPA 1631 (a method approved for the analysis of biota specifically) and careful scrutiny of the results of numerous quality control analyses, it is clear that results reported utilizing EPA 7471 are approximately 20% lower than would otherwise be reported by more accurate methods and it is "not applicable" to the analysis of fish tissues.

If accuracy is of concern when monitoring for mercury levels in fish, especially in support of consumption advisories, EPA 1631 is a superior method for the preparation and analyses of fish tissue samples for the determination of mercury concentrations.

Investigation into Low-Bias

Out of scientific and professional curiosity, a number of further experiments were performed in an attempt to isolate the potential cause(s) of the low-bias observed in the results of analyses of fish tissue samples by EPA 7471.

The method is approved for, and presumably adequate for, the analysis of soils, sediments, bottom deposits, and sludge-type materials for mercury concentrations, but it appears to be insufficiently robust for the determination of mercury concentrations in fish tissue samples, a distinctly different type of matrix than those listed above.

Due to the consistently precise results achieved by the method, as demonstrated by the low RPD of the numerous duplicate analyses, it was presumed that the issue did not involve a problem with the instrumentation or the detection of the analyte, but rather an insufficiently robust preparation procedure.

There are at least two potential causes for a low-bias related to the sample preparation procedure: incomplete dissolution of the sample tissues, or incomplete oxidation by potassium permanganate of the mercury released into solution.

Incomplete digestion may result in some of the organo-mercury complexes not dissolving into solution. Incomplete oxidation may result in some of the mercury in solution not converting to divalent mercury. Either of these conditions, alone or in conjunction, can lead to lower reported concentrations of mercury.

To investigate and possibly isolate the cause of the low-bias, another set of fish tissue samples were analyzed again by both methods, but with variations to the preparation procedures.

Tissue samples were collected from three freshwater fish and were prepared and analyzed in triplicate by modifications of methods EPA 7471 and EPA 1631 concurrently. Quality control samples for each method included the analyses of two MSs of the fish samples and an SRM, DORM-3 *Fish Protein* (National Research Council Canada, Institute for National Measurement Standards, Ottawa, Canada).

The preparation procedure for EPA 7471 was modified to increase the digestion time from 30 minutes to 8 hours and potentially improve the completeness of the digestion. The preparation procedure for EPA 1631 was modified to use potassium permanganate as the oxidizing reagent and potentially confirm that it completely converts all of the mercury in solution to divalent mercury.

The results of the analyses in triplicate by both of the modified preparation techniques, EPA 1631 (Figure 10) and EPA 7471 (Figure 11), demonstrate excellent precision. However, when the average of the results for each sample are compared (Figure 12), the results using the modification of EPA 1631 continue to be greater than the results using the modification of EPA 7471 (by approximately 20%).

Despite the extended digestion time (8 hours) performed in the modification of EPA 7471, the vessels still contained visible particulate matter and the recoveries of the matrix spikes partially reflect this (Figure 13). The recoveries of the SRMs using the modification of EPA 1631 (Figure 14) seem to indicate that the use of potassium permanganate as the oxidizing reagent is adequate to completely convert all of the mercury in solution to divalent mercury.

This evidence, while inconclusive, suggests that the type and volume of the particular acids used is responsible for the low-bias rather than a shorter digestion time or an incomplete oxidation of the sample.

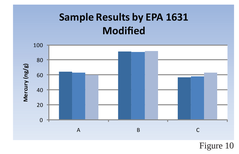

Figure 10

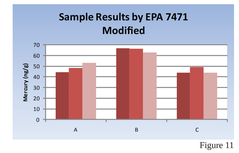

Figure 11

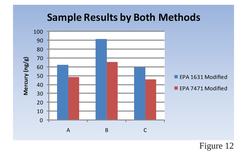

Figure 12

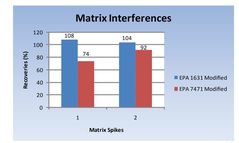

Figure 13

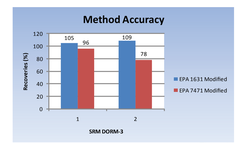

Figure 14