Results of Mercury Analyses Significantly Beyond Recommended Holding Times – Potential Implications for Mercury Holding Times in Biological Samples

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Introduction

The US EPA recommends a maximum holding time of 28 days for the analysis of mercury in biological tissue samples. However, this recommended holding time seems to be based on administrative, rather than technical, guidance. Some studies have indicated that mercury is stable in biological matrices for significantly longer time periods, particularly when the tissue is frozen or freeze-dried.

As part of the biological monitoring program for the Tennessee Valley Authority (TVA) Kingston Ash Recovery Project (KRP), mercury analyses were conducted for a variety of biological matrices, including whole-body fish, benthic invertebrates, bird eggs and nestlings, and mammal blood. These samples were archived in frozen storage following the initial analysis.

As part of a methods evaluation study, a subset of the project samples were reanalyzed for mercury by the original analytical method (SW-846 Method 6020) significantly beyond the 28-day holding time recommended by the US EPA. A comparison of the initial analysis and the reanalysis results generated using SW-846 Method 6020 was performed to evaluate the impact of long-term storage beyond the recommended holding time on the measured concentrations of mercury.

Data Set

133 samples were reanalyzed as part of this study.

- 30 fish carcass
- 28 fish filet
- 15 fish liver
- 15 fish ovary
- 15 fish gut and content
- 15 bird egg
- 15 tree swallow nestling

One fish carcass sample was excluded from the data analysis due to a very significant difference between the initial and reanalysis results which was an apparent outlier.

Fish ovaries were excluded from the data analysis because of the 15 samples, only 2 positive results were observed in the reanalysis. The initial results were not significantly different from the MDLs for the reanalyses; consequently, these results were in agreement.

For 5 samples (3 fish filet and 2 fish liver), mercury was detected in the initial analysis but was not detected in the reanalysis. The MDLs for the reanalysis were elevated compared to the initial, most likely due to limited sample mass available for the reanalysis. For 4 of the 5 samples, the initial result was less than the MDL for the reanalysis. For 1 of the 5 samples, the initial result was just above the MDL for the reanalysis. These results were excluded from the data analysis, but it should be noted that the initial and reanalysis results for these samples were in agreement.

As summarized in Table 1, a total of 112 samples were included in the data analysis.

Data Evaluation

Initial and reanalysis results were compared using project-specific acceptance limits for biological duplicate sample analyses. Results were considered to be acceptable when one of the following conditions was met:

- Where one result was < 5x the MDL, the difference was< 2x the RL
- Where both results were > 5x the MDL, the RPD was < 35%

In addition, the percent change from the initial result to the reanalysis result was determined. The change over time was evaluated for each reanalyzed sample.

Results and Discussion

Summary statistics for the dataset evaluated are presented in Table 1. As previously described, a few samples were excluded from the data analysis.

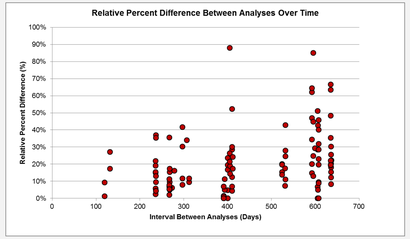
Table 1: Mercury Reanalysis Summary Statistics

Matrix	# Samples	Timeframe Between Initial and Reanalysis	% Acceptable	% Significantly Different	% Increasing Results	Average Increase	% Decreasing Results	Average Decrease	Average Relative Change (%)
Fish Carcass	29	405 - 599 days	86%	14%	21%	+67%	79%	-23%	-5%
Fish Filets	25	119 - 411 days	96%	4%	88%	+17%	12%	-3%	+14%
Fish Livers	13	266 - 278 days	100%	0%	31%	+9%	69%	-11%	-5%
Fish Gut and Content	15	236 - 410 days	87%	13%	80%	+20%	20%	-6%	+14%
Bird Eggs	15	635 - 636 days	80%	20%	27%	+22%	73%	-26%	-13%
Bird Nestlings	15	605 - 607 days	100%	0%	60%	+22%	40%	-25%	+3%
All Samples	*112*	*119 - 636 days*	*91%*	*9%*	*52%*	*+24%*	*48%*	*-20%*	*+3%*

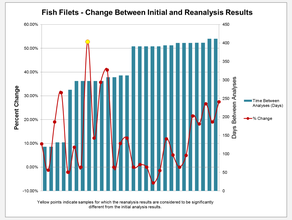
Results and Discussion (Cont'd)

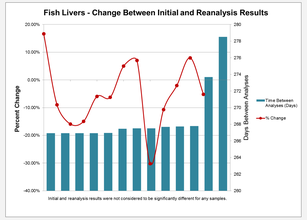
- Greater than 90% of the reanalysis results were acceptable (*i.e.*, within duplicate acceptance criteria) for the overall dataset. The lowest percentage of acceptable reanalysis results was observed for bird eggs. Bird eggs were reanalyzed at the greatest average interval following the initial analysis.
- The trend toward sample results increasing or decreasing following extended storage was highly variable between the different sample matrices. For the overall dataset, the samples were nearly evenly split, with 52% of results increasing an average of 24% and 48% of results decreasing an average of 20%. The average relative change across the dataset was an increase of 3%.
- In a few cases, the average relative change was biased due a single vastly higher or lower result in the reanalysis. It is likely that these differences were the result of sample heterogeneity, rather than the extended time held in frozen storage.

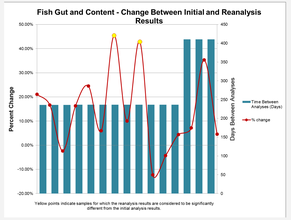
A plot of the relative percent difference (RPD) between the initial analysis and reanalysis over time revealed a general increase in imprecision over time. As the interval between analyses approached 600 or more days, a wider range of RPDs were observed. It should be noted that the RPD is a poor indicator of the comparability of low-concentration results (*i.e.*, reported near the MDL); accordingly, the reanalysis results may be considered acceptable (as previously defined) even when high RPDs are observed. For many of the samples reanalyzed following the longest intervals, the results were sufficiently close to the MDL that the RPD was not used to determine acceptability.

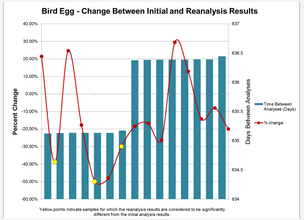


The variability between initial analysis and reanalysis results on a matrix-specific basis are presented.

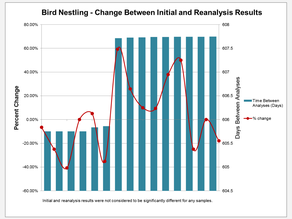








As presented above, the relative change between analyses was variable on a sample-specific basis. The data analysis did not reveal a clear trend toward an increase or decrease in results over time. An evaluation of the matrix-specific average relative change revealed an overall decrease in results some matrices (fish carcass, fish liver, and bird eggs) and an overall increase in results for other matrices (fish filets, fish gut and content, and nestlings). It appears that sample-specific conditions such as homogeneity and sample mass may have a greater impact on measured mercury concentrations than the holding time. Other studies have suggested that changes in sample moisture content over time may impact measured analyte concentrations.



Conclusions

This study examined mercury results generated for a variety of biological matrices held in frozen storage for an extended timeframe. Samples were reanalyzed by the same method between 119 and 636 days beyond initial analysis; these intervals significantly exceed the maximum holding time of 28 days for the analysis of mercury in biological tissue samples recommended by the U.S. EPA. Overall, greater than 90% of the observed reanalysis results were considered to be acceptable when compared to project-specific acceptance criteria for biological duplicate samples. The data did not reveal a clear trend for increase or decrease of mercury results among the various matrices. When examined as a whole, the dataset revealed a nominal 3% average increase; when compared to the method variability of ~10%, this change does not appear to be significant. Based on the dataset examined, it does not appear that extended frozen storage has a clear impact on mercury results; rather, sample-specific variables such as homogeneity may have a much greater impact.

Acknowledgement

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