Determining Methylmercury Concentrations in Mammals and Birds Utilizing Nondestructive Sample Collection Techniques

In contrast to naturally occurring sources, human activities have substantially increased the mobilization of mercury in the environment and are by most estimates responsible for more than half of all observed contamination issues, primarily as a consequence of energy production (i.e., bituminous coal combustion), which can release relatively significant quantities of elemental mercury vapor (Hg^0) into the atmosphere.

The rate at which mercury emissions then deposit on the earth's surface as oxidized divalent mercury (Hg^{2+}) is contingent on multiple complex factors(1), and while a substantial ratio may fall in the immediately surrounding area (Figure 1), emissions can also enter the upper atmosphere and be transported for months or longer before settling nearly anywhere on the planet.

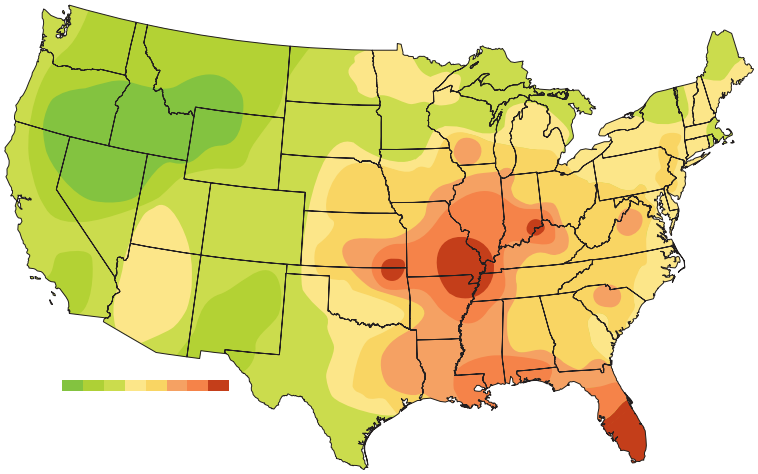

Figure 1. Estimated relative concentrations of mercury deposition across the continental United States based upon data provided by the National Atmospheric Deposition Program.

It is for this reason that mercury is considered a global pollutant: its reach includes the environments of areas far removed from the point of its original release, potentially impacting remote wilderness areas and the wildlife that inhabit them.

30th anniversary 1982 - 2012
BROOKS RAND LABS
MEANINGFUL METALS DATA®

Easily mobilized in water, divalent mercury can then reach larger aquatic ecosystems where the potential for mercury contamination to become an even more serious issue largely depends on whether the ecosystem where it settles is favorable to the conversion of inorganic mercury to the significantly more toxic organic form of methylmercury (CH_3Hg). Wetlands, low-alkalinity lakes, organic-acid rich systems, recently flooded areas, and streams where severe level fluctuations take place are particularly common areas for mercury methylation to occur.

With an average half-life in aquatic organisms of about two months, methylmercury can bioaccumulate within individual organisms and then biomagnify up trophic levels (Figure 2). Large piscivorous fish can have concentrations of methylmercury in their tissues easily exceeding a million times greater than the surrounding water.

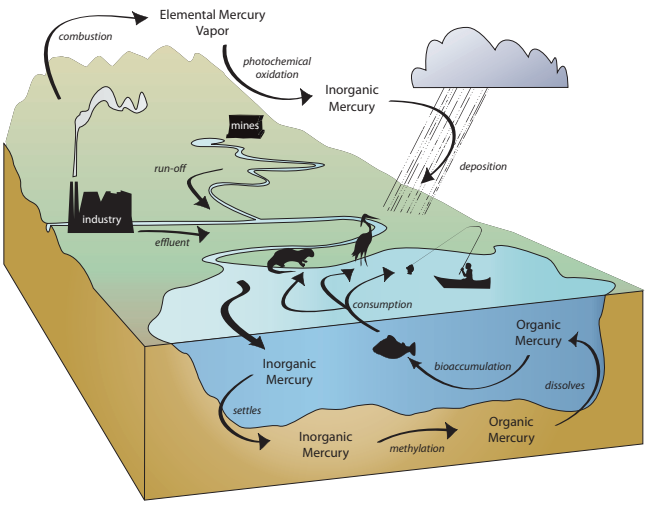

Figure 2. Environmental fate and transport of anthropogenic mercury.

The most prevalent means of human exposure to mercury pollution is through the consumption of fish containing elevated concentrations of methylmercury, but this is equally true for mammals and birds whose diets consist primarily of fish from mercury impacted environments.

The increasing quantities of mercury being introduced into the global environment have long been a matter of considerable concern for human health, but a growing body of research is demonstrating that mercury contamination can also seriously threaten the health and reproductive capabilities of wildlife.

For example, just last year researchers from the University of Florida published the results of a study in *Aquatic Toxicology* where they examined the effects of methylmercury exposure on white ibises (*Eudocimus albus*) over more than three years(2). They concluded that the results suggest chronic exposure to even low and environmentally-relevant levels of dietary methylmercury will alter hormone levels and may be a widespread mechanism by which reproduction is impaired in wild bird populations. Neurological damage, impaired growth and reproduction, and early mortality have also been observed in mammals exposed to relatively low concentrations of methylmercury.

Experimental

Primarily with the intent to protect human health, the edible tissues of wild game species (i.e., fish, birds, mammals) are routinely tested for low levels of methylmercury by those few laboratories capable of performing such highly sensitive and specialized analyses, but this inherently occurs in conjunction with the destruction of the animals in question.

When monitoring for methylmercury in nongame species, it is often desirable for a variety of obvious reasons to do so by collecting samples noninvasively and avoiding the destruction of the specimens. For example, during capture and release efforts related to wildlife conservation studies – fur or feather samples can often be collected without significant harm to the subject specimens.

However, the sample preparation methods that are typically employed in the analysis of tissue samples for methylmercury have been found to be far less effective when analyzing samples that contain dense keratin filaments (e.g., fur, feathers, nails, antlers), producing non-detect results that have little value in wildlife monitoring studies.

The most widely accepted technique for preparing tissue samples for the analysis of methylmercury – via a modification of EPA Method 1630 – involves an alkaline digestion of the sample using a solution of potassium hydroxide and methanol (KOH/MeOH), a technique capable of achieving a method detection limit as low as 1 ng/g(3), which is more than sufficiently sensitive to monitor for environmentally-relevant concentrations given sufficient sample mass.

Figure 3 shows example recoveries for a standard reference material (SRM), *NIST SRM-1946: Lake Superior Fish Tissue*, and sample matrix spikes (MS), and Figure 4 shows some example data for methylmercury concentrations in fish tissue using the alkaline digestion technique.

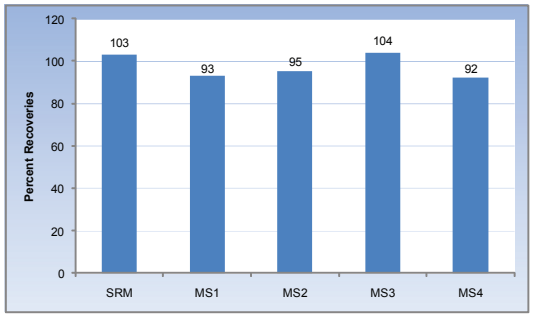

Figure 3. Standard Reference Material (SRM) and fish tissue Matrix Spike (MS) recoveries prepared by potassium hydroxide and methanol.

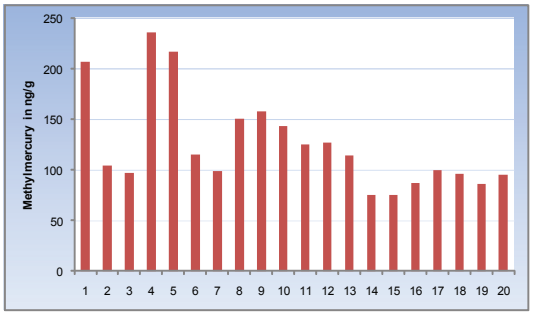

Figure 4. Example results of methylmercury concentrations in fish tissue samples prepared by potassium hydroxide and methanol.

As can be seen, the accuracy of this preparation and analytical method is well within the most stringent quality control criteria and most of the sample results for methylmercury concentrations easily exceed the method detection limit by several degrees of magnitude.

However, when this same method is used in the preparation of animal fur samples, none of the relevant quality control samples produce recoveries that are within anything resembling acceptable limits (Figure 5) and the majority of the sample results for methylmercury fall at or below the detection limit (Figure 6). The SRM used for confirmation of method accuracy in this analysis was *IAEA-086: Human Hair*.

These low recoveries are a clear indication that the alkaline preparation technique is inadequate to overcome the matrix interferences probably introduced by the keratin filaments found in the animal fur samples and that the methylmercury that may be present in the sample is not being effectively extracted for analysis.

These matrix interferences may be overcome by significantly diluting the preparations prior to analysis, but that can also increase the method detection limit to the extent that it exceeds environmentally-relevant levels, as will be demonstrated below.

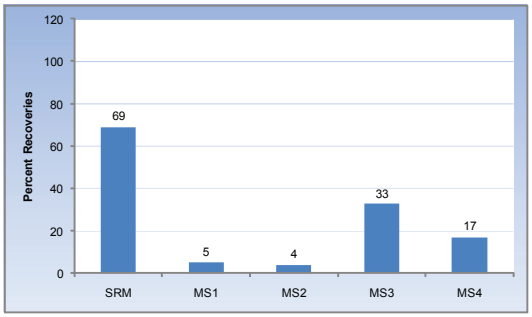

Figure 5. Standard Reference Material (SRM) and fur sample Matrix Spike (MS) recoveries prepared by potassium hydroxide and methanol.

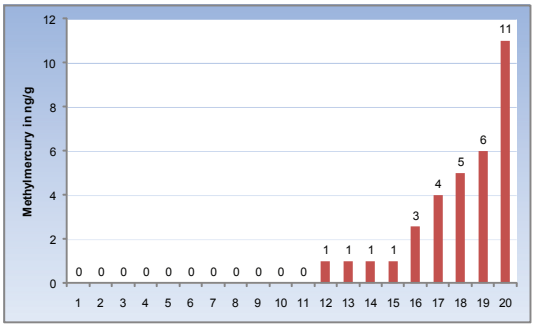

Figure 6. Example results of methylmercury concentrations in fur samples prepared by potassium hydroxide and methanol.

Recently developed for use in preparing composites of insect specimens (mosquitoes) for the determination of methylmercury(4), an acidic digestion technique using ultra-pure concentrated nitric acid was performed on the same animal fur and quality control samples shown in Figure 5, producing excellent recoveries for all of the relevant quality control samples (Figure 7).

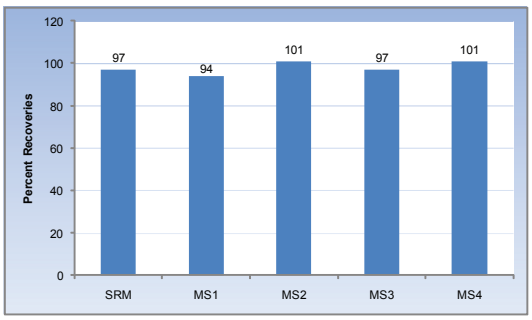

Figure 7. Standard Reference Material (SRM) and fur sample Matrix Spike (MS) recoveries prepared by nitric acid.

Furthermore, all of the results for methylmercury in the samples far exceeded the method detection limit (Figure 8). This method is also potentially more precise with an achievable detection limit as low as 0.1 ng/g, which may be a significant advantage when only extremely limited sample masses can be collected.

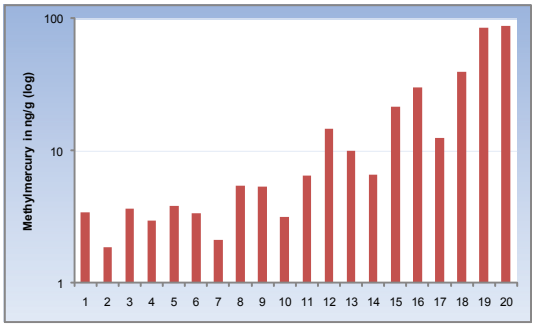

Figure 8. Example results of methylmercury concentrations in fur samples prepared by nitric acid.

A quick comparison of the results produced by both sample preparation techniques demonstrates rather conclusively the superiority and relevancy of the data when using the acidic digestion method (Figure 9).

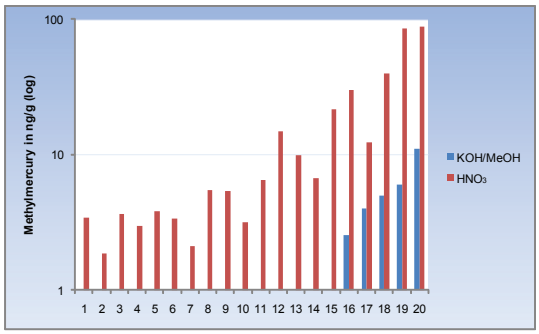

Figure 9. Comparison results of methylmercury concentrations in fur samples prepared by both methods.

The same comparative procedures were also used on three bird feather samples to demonstrate the potentially wide application of this method to researchers of wildlife impacted by mercury contamination (Figure 10).

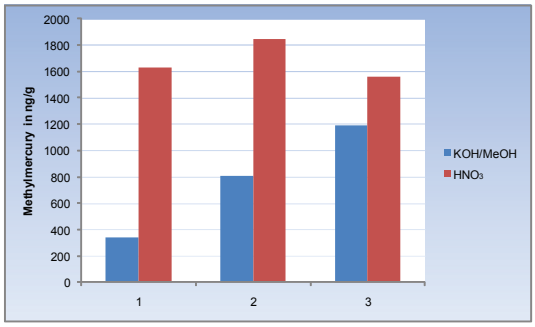

Figure 10. Comparison results of methylmercury concentrations in feather samples prepared by both methods.

Conclusions

The biological impact of mercury contamination (and methylmercury in particular) upon wildlife is still a matter of debate and requires significant further research, especially the relationship of methylmercury concentrations in fur or feathers to total body concentrations, but the analytical techniques available to wildlife researchers desiring to further explore these issues has only improved, given that the laboratory which performs these analyses has sufficient expertise to provide the quality of data necessary to distinguish background levels from the impact of contamination.

Nondestructive sample collection techniques for the determination of relative methylmercury concentrations in mammals and birds can provide scientists involved in wildlife conservation studies an excellent tool to assess the true impact of mercury contamination in the subject species without diminishing the population in the process.

References

1. Lyman, S.; Jaffe, D. Formation and fate of oxidized mercury in the upper troposphere and lower stratosphere. *Nature Geoscience*. **2011**, DOI: 10.1038/ngeo1353.
2. Jayasena, N.; Frederick, P.; Larkin, I. Endocrine disruption in white ibises (*Eudocimus albus*) caused by exposure to environmentally relevant levels of methylmercury. *Aquatic Toxicology*. **2011**, 105, 321-327.
3. Bloom, N. S. On the chemical form of mercury in edible fish and marine invertebrate tissue. *Can. J. Fish. Aquat. Sci.* **1992**, 49, 1010-1017.
4. Hammerschmidt, C. R.; Fitzgerald, W. F. Methylmercury in Mosquitoes Related to Atmospheric Mercury Deposition and Contamination. *Environ. Sci. Technol.* **2005**, 39, 3034-3039.

Brooks Rand Labs
3958 6th Ave NW
Seattle WA 98107 USA
206-632-6206
www.brooksrand.com

30th anniversary 1982 - 2012 BROOKS RAND LABS MEANINGFUL METALS DATA®