Stability of Mercury Concentrations in Frozen Avian Blood Samples

Bulletin of Environmental Contamination and Toxicology

ISSN 0007-4861
Volume 86
Number 2

Bull Environ Contam Toxicol
(2010) 86:159-162
DOI 10.1007/
s00128-010-0164-0

Springer

Bull Environ Contam Toxicol (2011) 86:159–162
DOI 10.1007/s00128-010-0164-0

Stability of Mercury Concentrations in Frozen Avian Blood Samples

Claire W. Varian-Ramos · Anne M. Condon · Kelly K. Hallinger · Kjarstin A. Carlson-Drexler · Daniel A. Cristol

Received: 5 November 2010 / Accepted: 20 November 2010 / Published online: 5 December 2010

Abstract It is unclear whether mercury concentration in wildlife tissues changes appreciably after lengthy frozen storage. To test whether such freezer-archived samples are stable, small (~10–50 μL) avian blood samples stored in capped glass capillary tubes were analyzed for total mercury concentration, and then reanalyzed after being frozen for up to 3 years. Mercury concentrations increased 6% on average over the 3 year period, but time spent frozen explained only 11% of the variation between measurements. This small amount of change suggests that archived blood samples remain useful for at least several years.

Keywords Avian blood · Temporal stability · Mercury · Tissue storage

Many studies of mercury concentrations in vertebrate animals now make use of tissues that can be sampled non-lethally, such as feathers, fur or blood. As researchers continue to refine our knowledge of the effects of mercury on wildlife, it is important to determine whether samples that have been stored for extended periods are still useful. Long holding times allow for samples to be archived for later analysis prompted by new discoveries, funding, or research questions.

C. W. Varian-Ramos (✉) · K. K. Hallinger · K. A. Carlson-Drexler · D. A. Cristol
Biology Department, College of William and Mary, P.O. Box 8795, Williamsburg, VA 23187-8795, USA
e-mail: cwramos@wm.edu

A. M. Condon
U.S. Fish and Wildlife Service, 6669 Short Lane, Gloucester, VA 23061, USA

The maximum length of time between sample collection and mercury analysis that will result in an accurate measure of mercury concentration is not well described in the literature. Most studies have focused on storage of fish tissues (De Boer and Smedes 1997; Horvat and Byrne 1992; Peterson et al. 2007) or samples that have already undergone some degree of processing (Devai et al. 2001; Parker and Bloom 2005). Studies describing long-term storage of mammalian (human) blood samples include only relatively large samples (1–5 mL) (Horvat and Byrne 1992; Liang et al. 2000; Spěváčková et al. 2004) which may differ from the smaller samples typically collected from birds and other wildlife species. Currently there are recommendations for the maximum length of time that fish tissues can be stored before mercury analysis, ranging from 28 days to 4 years (Peterson et al. 2007; USEPA 1995) and we are unaware of such published recommendations for other birds or other wildlife.

Much research on mercury contamination in avian blood has occurred on the South River in Virginia (e.g., Brasso and Cristol 2008; Condon and Cristol 2009; Hallinger et al. 2010). However, there is no literature describing the effects of holding time on mercury concentration in bird blood. The objective of this study was to determine whether there was any change in the mercury concentrations of avian blood samples after various lengths of time in a −20°C freezer for up to approximately 3 years.

Materials and Methods

Blood samples were collected from bird species at mercury-contaminated sites located along the South River, Augusta and Rockingham counties, Virginia, and reference sites in the same watershed on the South, Middle or North

Rivers during the spring and summer breeding seasons of 2005–2008 (map of study sites in Cristol et al. 2008). Fourteen species were sampled: barn swallow (*Hirundo rustica*), belted kingfisher (*Ceryle alcyon*), Carolina chickadee (*Poecile carolinensis*), Carolina wren (*Thryothorus ludovicianus*), eastern bluebird (*Sialia sialis*), eastern phoebe (*Sayornis phoebe*), field sparrow (*Spizella pusilla*), gray catbird (*Dumetella carolinensis*), great crested flycatcher (*Myiarchus crinitus*), house wren (*Troglodytes aedon*), indigo bunting (*Passerina cyanea*), northern rough-winged swallow (*Stelgidopteryx serripennis*), tree swallow (*Tachycineta bicolor*), and tufted titmouse (*Baeolophus bicolor*). Blood (10–50 μL) was sampled from the brachial (a.k.a. cutaneous ulnar) vein using a 26-guage needle (Becton–Dickinson, Franklin Lakes, NJ) to make a small opening through which blood was collected into 75 μL heparinzed micro-capillary tubes (Fisher Scientific, Pittsburgh, PA) capped with critocaps (Oxford Labware, St. Louis, MO). The capillary tubes were placed in sealed 10 mL vacutainers (Becton–Dickinson, Franklin Lakes, NJ) to prevent breakage, and then in a zipping plastic bag for storage at −20°C prior to analysis. The samples were initially analyzed within a median of 72 days (range: 2–542 days) of sample date and duplicate samples remained frozen for up to 1,140 additional days, at which point they were reanalyzed using the same equipment that was used for the initial measurement. Sample selection for reanalysis was not based on anomalous first measurement or sample volume; rather, samples were selected specifically for this study, randomly with the constraint of availability of two samples from same bird and an upper limit on number of samples with same number of years in freezer. A total of 209 paired samples were analyzed: 62 samples within a year, 58 separated by 1–2 years, 69 separated by 2–3 years, and 20 samples >3 years apart. In addition, when more than two tubes of blood were available from an individual bird, duplicate samples were run on the same day for quality control. These duplicates provided an estimate of the variation between replicates due to variability in the bird's blood stream, machine error and other factors unrelated to frozen storage.

Samples were analyzed for total mercury at the College of William and Mary between 2006 and 2010. We used atomic absorption spectroscopy with a Milestone DMA-80 direct mercury analyzer (Shelton, CT, USA). The DMA-80 was calibrated using known standards according to machine specifications approximately every 2 months throughout the study period or more often when necessary to keep standard reference material values within 5% of certified values. A sample blank (no sample or container), methods blank (empty container), duplicate, and two samples of each standard reference material (DORM-3 and

Table 1 Quality assurance for mercury analyses within years

	2007	2008	2009	2010	Total
Average method detection limit ± SD (μg/g)	0.004 ± 0.002	0.005 ± 0.002	0.003 ± 0.001	0.004 ± 0.001	0.004 ± 0.002
Duplicate sample analysis					
Number of pairs	64	108	21	5	198
Mean relative % diff. ± SD	3.3 ± 4.3%	2.7 ± 2.6%	3.7 ± 4.9%	3.0 ± 5.8%	3.0 ± 3.6%
Standard reference materials					
DORM-3 bottle 1					
Certified value (μg/g)	0.409	0.409	0.409		0.409
Number of replicates	152	551	218		921
Mean value ± SD	0.387 ± 0.005	0.408 ± 0.009	0.410 ± 0.023		0.405 ± 0.016
DORM-3 bottle 2					
Certified value (μg/g)			0.382	0.382	0.382
Number of replicates			190	36	226
Mean value ± SD			0.390 ± 0.017	0.384 ± 0.015	0.389 ± 0.017
DOLT-3					
Certified value (μg/g)	3.37	3.37	3.37		3.37
Number of replicates	152	552	218		922
Mean value ± SD	3.39 ± 0.08	3.32 ± 0.09	3.40 ± 0.11		3.35 ± 0.10
DOLT-4					
Certified value (μg/g)			2.58	2.58	2.58
Number of replicates			190	36	226
Mean value ± 95% CI			2.57 ± 0.08	2.52 ± 0.03	2.56 ± 0.07

DOLT-3 or DOLT-4) were run before and after every batch of approximately 20 samples. Two separate capillary tubes of blood from the same collection date of the same bird run on the same day were considered duplicate samples. Minimum detection limit was 0.002–0.009 μg/g over the entire period of the study. Recovery of total mercury was 101.1 ± 3.7% for DORM-3 (bottle 1), 98.4 ± 4.2% for DORM-3 (bottle 2), 100.6 ± 3.0% for DOLT-3, and 100.6 ± 2.6% for DOLT-4 (Table 1).

To detect any trend in mercury concentration over time we used a linear regression. The intercept was forced through zero because the average difference between any two samples run on the same day should, in theory, be zero. We also present data with the samples grouped by the number of years between measurements using an ANOVA to detect any differences in magnitude of the change in concentration between years. In addition, in each year we compared the proportion of samples that had a positive change in mercury concentration to the proportion with a negative change. We used a sign test to determine if the proportions differed from the expected 50:50. All mercury concentrations are presented as wet weight (μg/g) values.

Results and Discussion

Because we used samples from contaminated and reference sites there was a wide range of mercury values represented: 0.04–8.39 μg/g. On average, mercury concentration increased by 5.9 ± 14.8% (mean ± SD) between the first and second measurement, regardless of number of years in storage. The change in mercury concentration appeared to increase with increasing time between measurements (i.e., significant slope, $N = 209$, $F_{1,208} = 26.04$, $p < 0.001$, Fig. 1); however time between measurements explains only a small proportion of the variation in the change between measurements (i.e., low $R^2 = 0.11$). Duplicate samples run on the same day differed by an average of 3.0 ± 3.6% (mean ± SD, Table 1), but this variance was not in a particular direction, unlike the change due to storage.

The magnitude of change in mercury concentration appeared to differ depending on the number of years between measurements (i.e., 0–1, 1–2, 2–3, >3 years; $N = 209$, $F_{3,205} = 2.77$, $p = 0.043$), however, after using Tukey's adjustment for multiple comparisons, this trend was not significant (Fig. 2). Over the course of the study, significantly more samples increased in mercury value than would be expected by chance ($N = 147/209$, $p > 0.001$).

Long-term storage of blood samples resulted in a modest increase in concentration of mercury in these samples, but the increase remained under 10% after more than 3 years of storage and was not highly dependent on duration of storage. This apparent increase of mercury in the samples more likely represents a loss of water weight, as the mercury concentrations were measured using the wet weight of the blood. Additionally, the increase in mercury concentration may not have been consistent over time. Grouping the samples by number of years stored did not reveal a continuing increase in mercury concentration. In fact, there was no significant difference between any of the year groupings. It may be that water was lost relatively rapidly from the blood near the ends of the capillary tubes in which the samples were stored, but blood in the middle of the capillary tube was then protected from further evaporation. Reanalyzing samples again after an even longer period of storage (5–10 years) will reveal whether further evaporation occurs over longer time scales. It is important to note

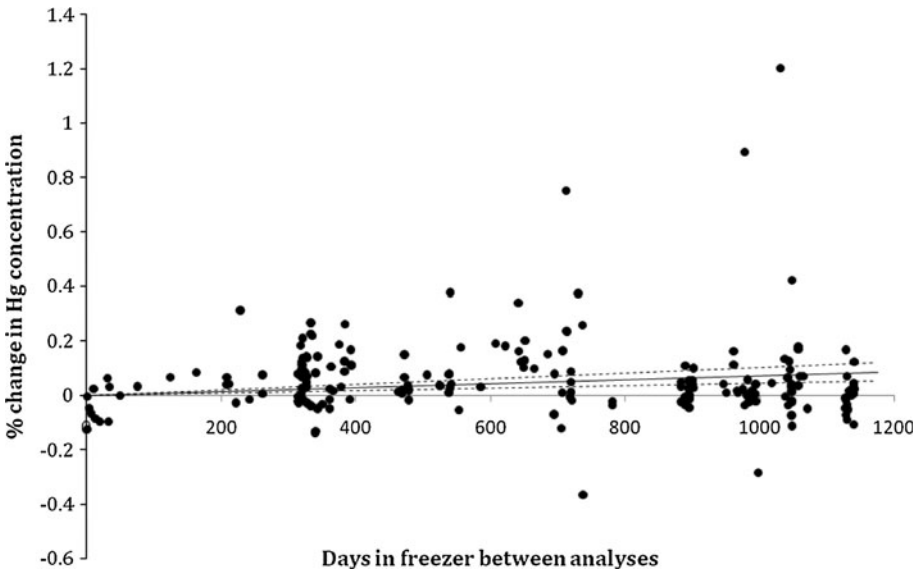

Fig. 1 The % change in mercury between duplicate samples plotted against time between analyses (days in freezer). The *solid line* represents the linear regression. The *dotted lines* are the 95% C.I. of the regression line

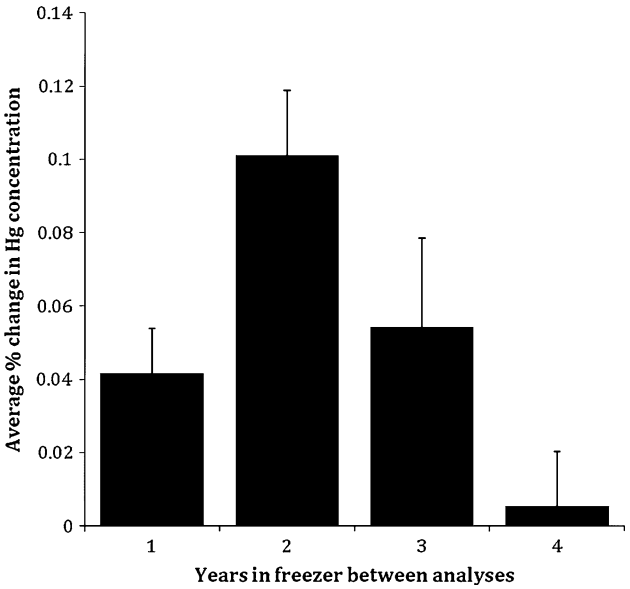

Fig. 2 Average change in mercury by number of years in storage. *Error bars* are one standard error

that samples had already been frozen for typically 2–3 months before the initial analysis, so the difference in mercury concentrations between first and second measurements was not the result of water loss upon freezing.

Regardless of the mechanism, the mercury concentration of these small-volume (10–60 μL) blood samples remained relatively stable over time. Samples exhibited an average increase in mercury concentration of only 6% after multiple years in a standard household freezer, which may be an acceptable level of error for many studies. If this amount of change is deemed unacceptable for a particular study, freeze drying the samples prior to analysis would eliminate the problem, if the increase is indeed caused by evaporation from the sample as we hypothesize. However, freeze drying of some tissues, including blood, has been shown to result in losses of mercury compounds of 5% or more (LaFleur 1973; Horvat and Byrne 1992). Storage time explained little of the variation between samples, and samples stored for >3 years were within 10% of original concentrations. Thus, we recommend that researchers regularly archive samples of bird blood for future study, and occasionally check stability of their mercury samples as demonstrated here.

Acknowledgments We thank the South River Science Team. Funding was provided by E. I. DuPont de Nemours and Company, The Office of Vice Provost for Research at the College of William and Mary, National Science Foundation Grant UBM 0436318, and the William and Mary Undergraduate Science Education and Research Program sponsored by the Howard Hughes Medical Institute.

References

Brasso RL, Cristol DA (2008) Effects of mercury exposure on the reproductive success of tree swallows (*Tachycineta bicolor*). Ecotoxicology 17:133–141

Condon AM, Cristol DA (2009) Feather growth influences blood mercury level of young songbirds. Environ Toxicol Chem 28: 395–401

Cristol DA, Brasso RL, Condon AM, Fovargue RE, Friedman SL, Hallinger KK, Monroe AP, White AE (2008) The movement of aquatic mercury through terrestrial food webs. Science 320:335

De Boer J, Smedes F (1997) Effects of storage conditions of biological materials on the contents of organochloride compounds and mercury. Mar Pollut Bull 35:93–108

Devai I, Delaune RD, Patrick WH Jr, Gambrell RP (2001) Changes in methylmercury concentration during storage: effect of temperature. Org Geochem 32:755–758

Hallinger KK, Cornell KL, Brasso RL, Cristol DA (2010) Mercury exposure and survival in free-living tree swallows (Tachycineta bicolor). Ecotoxicology (currently available online) (In Press)

Horvat M, Byrne AR (1992) Preliminary study of the effects of some physical parameters on the stability of methylmercury in biological samples. Analyst 117:665–668

LaFleur PD (1973) Retention of mercury when freeze-drying biological materials. Anal Chem 45:1534–1536

Liang L, Evens C, Lazoff S, Woods JS, Cernichiari E, Horvat M, Martin MD, DeRouen T (2000) Determination of methyl mercury in whole blood by ethylation-GC-CVAFS after alkaline digestion-solvent extraction. J Anal Toxicol 24:328–332

Parker JL, Bloom NS (2005) Preservation and storage techniques for low-level aqueous mercury speciation. Sci Total Environ 337: 253–263

Peterson SA, Peck DV, Van Sickle J, Hughes RM (2007) Mercury concentration in frozen whole-fish homogenates is insensitive to holding time. Arch Environ Contam Toxicol 53:411–417

Spěváčková V, Korunová V, Čejchanová M, Vobecký M (2004) Sampling procedure and a radio-indicator study of mercury determination in whole blood by using an AMA 254 atomic absorption spectrometer. Anal Bioanal Chem 380:346–350

United States Environmental Protection Agency (1995) QA/QC guidance for sampling and analysis of sediments, water, and tissues for dredged material evaluations—chemical evaluations. EPA 823/B/95/001. US Environmental Protection Agency, Washington