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Dried blood spots for estimating mercury exposure in birds☆

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ABSTRACT

Mercury (Hg) is a pervasive environmental contaminant that can impair avian health, consequently there is a need to gauge exposures. Bird blood provides a measure of recent dietary exposure to Hg, but blood collection and storage can be complex and costly. Dried blood spots (DBS) may help overcome challenges of whole blood analyses, therefore, this study aimed to develop and validate a novel method to assess Hg exposure in birds using DBS. First, accuracy and precision of blood Hg concentrations for entire DBS and DBS punches were determined for white leghorn chicken (*Gallus gallus domesticus*) dosed with methylmercury (MeHg) via egg injection. Next, we investigated Hg stability in chicken DBS subjected to time, temperature, and humidity treatments. Lastly, we applied the method to DBS created using standard field methods from zebra finch (*Taeniopygia guttatato*) in the laboratory and American golden-plover (*Pluvalis dominica*) sampled in the field. All samples were analyzed for total Hg (THg) using direct Hg analysis. Accuracy was determined by comparing DBS concentrations with those of corresponding whole blood and reported as percent recovery. Accuracy for entire chicken DBS was 101.8 ± 5.4%, while DBS punches revealed lower recovery (87.7 ± 4.0 to 92.4 ± 4.1%). There was little effect of time, temperature, and humidity storage treatments on Hg concentrations of DBS, with mean DBS THg concentrations within ±8% of whole blood (n = 10 treatments). For zebra finch, DBS punches were more accurate (93.7 ± 9.7%) compared to entire DBS (126.8 ± 19.4%). While for American golden-plover, entire DBS resulted in the most accurate THg concentrations (111.5 ± 7.6%) compared to DBS punches (edge: 115.4 ± 18.9%, interior: 131.4 ± 16.1%). Overall, results indicate that DBS analysis using direct Hg analysis can accurately evaluate Hg exposure in birds.

1. Introduction

Mercury (Hg) is a widespread contaminant mobilized in the environment mainly from anthropogenic activities (UNEP, 2013). In birds and other wildlife, chronic exposure can cause sub-lethal effects which may potentially lead to population level impacts (Evers et al., 2008; Scheuhammer et al., 2011; Seewagen, 2010). Accordingly, efforts to reduce anthropogenic emissions, such as the UN Minamata Convention on Mercury, are essential. Evaluating the effectiveness of this Convention (i.e., Article 22) will require the use of exposure science to assess and monitor Hg pollution across the landscape and over time (Evers et al., 2016; Gustin et al., 2016).

Birds are considered effective bioindicators of Hg exposure since they are widespread and tend to have well-defined home ranges during the breeding season. Furthermore, birds have the potential to provide vital information about Hg contamination in remote or under represented regions. Birds assimilate and accumulate Hg rapidly in multiple tissues (Bearhop et al., 2000), including blood, which can be sampled with minimum risk to populations. Blood is a particularly useful biomarker of recent Hg exposure, as studies on birds fed methylmercury (MeHg) dosed diets demonstrate that blood Hg concentrations markedly increase within the first weeks of dosing (Bearhop et al., 2000; Bennett et al., 2009; Fournier et al., 2002). Researchers have recently started using large-scale datasets of blood or blood-equivalent total Hg (THg) concentrations of birds to determine Hg contamination and risk throughout North America (Evers et al., 2011; Jackson et al., 2015; Ackerman et al., 2016).

Blood sampling of both hatchling and adult birds generally does not result in any lasting effects on the sampled individual when conducted using established protocols (Sheldon et al., 2008) though there are notable concerns. Blood sample volume should be limited to no more than 1% of the birds' body weight, though this volume should be reduced when possible, especially during energetically demanding time periods (Fair et al., 2010). In small birds,

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this volume restriction can reduce the availability of samples for Hg analysis, since the limited blood sampled may be prioritized for other uses. Established blood collection methods can require bulky supplies such as blood storage tubes, coolers, and ice packs, which may be burdensome to transport to remote locations. After collection, blood samples should be stored frozen with limited freeze-thaw cycles to reduce the possibility of Hg loss or changes in concentration due to a loss in water content (Horvat and Byrne, 1992; Liang et al., 2000; Varian-Ramos et al., 2011). This may require the availability of a freezer or cryoshipper during sampling and for the samples to be shipped frozen. For samples collected at remote locations, equipment and shipping costs may be prohibitive or logistically unfeasible. In summary, while large-scale, multi-species Hg exposure assessments in birds are increasing, expanding these assessments to remote or resource-limited regions may be hindered by complex and costly blood collection and storage methods.

Developing a simple, accurate, and cost-effective technique for collecting bird blood in the field will allow for Hg exposure assessments at remote, resource-limited locations where data are currently lacking. Dried blood spots (DBS) are a method for collecting blood by applying it to specialized filter paper and allowing it to air dry. Dried blood spots developed in the 1960s to detect metabolic diseases in newborns (Guthrie and Susi, 1963), are still used today as part of newborn screening efforts (Mei et al., 2001). In addition, more recent studies have used DBS to determine blood concentrations of toxic and essential elements in both humans (Basu et al., 2017; Chaudhuri et al., 2008) and birds (Lehner et al., 2013; Shlosberg et al., 2012). Avian blood sampling for DBS can be carried out according to standard blood collection methods, and can, therefore, be done safely without increasing bird handling time. Small blood volumes are used to create DBS, with each spot consisting of $\leq$60 μL of blood (approximately equivalent to $\leq$60 mg of blood wet weight). Furthermore, DBS can be easily sub-sampled via punches. This may allow for accurate evaluation of blood Hg concentrations and for the remaining portion of the DBS to be analyzed as replicate samples or be analyzed for additional measurements including other contaminants or hormones such as corticosterone (Doody et al., 2008; Rector et al., 2012). Samples may also be archived for later analyses. Additionally, loss of blood water content during sample storage, which can lead to changes in Hg concentrations for whole blood, is not a concern when using DBS. Standardized filter paper used for DBS is small and light-weight, and during collection, DBS samples likely do not need to be kept cold or stored frozen. This can reduce cost, weight, and the number of blood sampling supplies needed during field collection, as well as shipping costs.

Dried blood spot use has been standardized for a variety of human health related analyses, and Shlosberg et al. (2011) attempted to establish collection, storage, and analysis methods of avian DBS for a variety of toxicants, including Hg. However, Shlosberg et al. (2011) do not describe these methods in detail or provide information on the accuracy of these methods for determining blood Hg concentrations in birds. The overall objective of this study was to develop and validate a method to assess THg exposure in birds (and by extension, fish and wildlife) using DBS. There were 3 specific aims of this study (Fig. 1): Aim 1) develop an accurate and precise method to determine blood THg concentrations using entire DBS and DBS punches from a model avian species, white leghorn chicken (*Gallus gallus domesticus*); Aim 2) investigate the stability of DBS THg concentrations by exposing laboratory-created DBS from chickens to a variety of experimental time, temperature, and humidity treatments in a laboratory setting; and Aim 3) apply the developed method to DBS created with blood collected using standard field methods from zebra finch (*Taeniopygia guttatato*) dosed with MeHg in the laboratory and American golden-plover (*Pluvalis dominica*) sampled at an Arctic field site. Overall, this research intends to provide a method to help reduce logistic burdens and costs of collecting, storing, shipping, and analyzing field-collected avian blood samples for Hg concentrations.

2. Materials and methods

2.1. General overview

A diagrammatic overview of the aims of this study is provided (Fig. 1). In this section, we describe methodological details pertaining to the study species, sample collection, creating and sub-sampling DBS, and THg analysis. The subsequent Methods sections provide added details on each of the aims.

2.2. Study species and sample collection

For Aims 1 and 2, blood samples were obtained from a model avian species, white leghorn chicken. Fertilized chicken eggs (Couvoir Simetin, Inc., Mirabel, QC, Canada) were dosed with MeHg by egg injection methods described in Rutkiewicz and Basu (2013) to result in a dose of 3.2 microgram per gram (μg g^{-1}) egg. Eggs were then incubated at 37 °C and 65% humidity, and automatically turned every 45 min. At 14 d post-hatch, chickens were euthanatized by decapitation and whole blood samples were subsequently collected, mixed thoroughly, and stored in trace metal grade K_2EDTA (di-potassium ethylenediaminetetraacetic acid)-coated vacutainers (BD, Mississauga, ON, Canada) at −20 °C until needed.

For Aim 3.2, nestling zebra finches, bred from an existing captive population, were dosed with MeHgCl dissolved in double deionized water from 1 d until 21 d post-hatch at Simon Fraser University Department of Biological Sciences (Morran, 2016). Each day, nestlings were given 0.5 μL per g of body weight of a control (water only), a low dose (~60 μg g^{-1}), or a high dose (~150 μg g^{-1}) solution. Whole blood was collected at 22 d post-hatch in 2 heparinized capillary tubes via puncture of the brachial vein. The blood from one capillary tube per bird was transferred to individual 1.5 mL polypropylene tubes and stored at −20 °C until analyzed for THg concentrations. The second capillary tube was used to create DBS immediately after collection.

Additionally, for Aim 3.3, adult American golden-plover were captured (Bylot Island in Nunavut, Canada) during the 2015 breeding season, and whole blood samples were collected using standard protocols (Evers, 2008). Blood was collected in heparinized capillary tubes (~50 μL of blood per tube, <1% of body weight, Drummond Scientific Company, Broomall, PA, USA) via puncture of the brachial vein using a small gauge needle. Whole blood samples were collected in 1 capillary tube, sealed firmly on both ends with Critocaps (Leica Microsystems, Inc., Concord, ON, Canada), and placed in a labeled 6 mL plastic vacutainer (BD, Franklin Lakes, NJ, USA) to prevent breakage. During field collection, these samples were stored in a cooler with ice packs and kept in cold conditions in the permafrost for <2 weeks, until transferred to a freezer for the remainder of the breeding season. A second capillary tube was collected and was immediately used to create DBS in the field. Whole blood samples were shipped to McGill University on ice in coolers using 1 d shipping and upon arrival stored frozen (−20 °C), while DBS were shipped and stored at ambient temperature until analysis.

This research utilized model avian species, white leghorn chicken and zebra finch, dosed with MeHg in the laboratory in order to reduce costs, supplies, and personnel required to sample wild birds, as well as to reduce the overall number of individual