

Project Title: A Proposal to Develop the Rural Alaska Monitoring Program (RAMP), to Assess, Monitor, Model, and Adapt to the Environmental Health Impact of Climate Warming in Rural Alaska

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Abstract

The Arctic, including Alaska, has warmed significantly over the last five decades, with widespread changes in every region, particularly in Alaska's Arctic slope, north of the Brooks Range. Prominent changes include changes of ocean temperature, increase in permafrost temperature in many regions, warmer winter seasons, with longer and warmer snow-free seasons, warmer freshwater temperatures, movement of plant and wildlife species previously found in more southern regions of Alaska into the Arctic slope region, changes in summer and winter ranges of terrestrial mammal species, and the extension of more southern host species with their zoonotic pathogens into more northern regions of Alaska and Canada. These changes have resulted in new human and wildlife health threats in the Arctic slope region. The small population and remote location of rural Alaskan villages has always meant that rural Alaska residents have continued to utilize traditional subsistence wildlife species to a greater extent than any other US population, both for cultural reasons, and the high expense of imported western foods.

The identification of heavy metals such as mercury, and highly persistent lipophilic anthropogenic contaminants in the circumpolar food chain of all Arctic countries has raised awareness in wildlife scientists, and human health authorities on the need to better understand the possible climate-mediated influence on atmospheric and ocean transport mechanisms on the exposure of biota, including humans, in the Arctic. Certain contaminants are known to interfere with immune response in both humans and wildlife.

We developed a village climate and health impact assessment tool; identified climate change vulnerabilities for the Native Village of Selawik and developed a climate adaptation strategy; developed biomonitoring tools to assess wildlife exposure to zoonotic pathogens and

contaminants; created capacity in village residents to utilize these tools on hunter-killed wildlife; established laboratory support to analyze specimens obtained in village monitoring programs; and have encouraged the wide-spread adoption of these tools to create the Rural Alaska Monitoring Program (RAMP). The goals of RAMP are continuation of use of the traditional food species, information to reduce exposure to the existing and emerging zoonotic pathogens (zoonotic pathogens are microorganism causing diseases in animals which can also infect humans), reduction of exposure to contaminants, improved data for wildlife and human health authorities, and improved understanding on climate-influenced transport of contaminants and movement of zoonotic pathogens.

Background

The Alaska Native Tribal Health Consortium (ANTHC)

ANTHC is a non-profit tribal organization employing over 1900 people in Anchorage and other communities. ANTHC has an administrative unit, the Consortium Business Support Division that fully supports a wide variety of fiscal, contracting, compliance, personnel, and legal services. The ANTHC includes the thirteen largest Alaska Native regional tribal health corporations, with a representative of each forming the ANTHC Board of Directors, and several smaller regional tribal health corporation that, together, provide comprehensive health care for the 135,000 Alaska Natives (AN) living in Alaska. ANTHC is financed partly by the congressional appropriations for the Indian Health Service, and partly by other forms of medical insurance collections. Almost all research activities are grant supported. The tribal health system has a series of small regional community hospitals, and a 155 bed tertiary care center in Anchorage. Each of the regional tribal health corporations has medical, social, community health, and environmental services that provide assistance and services to the villages in the

region. ANTHC has centralized statewide technical support and consultative services available to the regional corporations. These include services from the ANTHC Division of Community Health Services (DCHS), and the Division of Environmental Health and Engineering (DEHE), and the Alaska Native Medical Center, described above.

The importance of traditional foods, and the concerns of safety of the traditional diet in the context of the warming climate, has resulted in the creation of the Center for Climate and Health, within the Division of Community Health Services, and the goals of this grant are a direct result of that concern.

Goals

The goal of this grant is the creation of village-based environmental monitoring in the villages of the Arctic LCC region. The Alaska Native Tribal Health Consortium (ANTHC) Rural Alaska Monitoring Program (RAMP) is based on village-specific, climate-sensitive environmental health threat assessments. The assessment process has components designed to gather data on subsistence species that will enable four specific outcomes:

1. Continued use of the subsistence diet, with the cultural, public health and economic benefits that are characteristics of that diet.
2. Development of village-specific adaptation strategies that will allow reduction of exposure to contaminants and zoonotic pathogens, facilitate identification of new pathogens, and allow the monitoring of trends in contaminant exposure and seroprevalence of existing zoonosis.
3. Development of data on trends in contaminant and pathogen prevalence in local and transboundary species for use by tribal, regional, state, federal and international wildlife management agencies.

4. The eventual merging of local weather and contaminant/pathogen trend data to enhance development of better modeling tools for predicting the impact of climate regime change on the movement of contaminants and pathogens in the circumpolar north.

Project Justification

The warmer seasons have created an environment in tundra water sources that favors bacterial methylation of Hg deposited by airborne transport from coal-fired power plants in Asia, and also released from melting snow and melting permafrost⁽¹⁾. In addition, warmer and longer ice-free seasons have resulted in cyanobacterial blooms in fresh and estuarine waters of the Chukchi Sea region, creating a risk of cyanobacterial toxin (microcystin) in village source water, as well as marine food species⁽²⁾. Neither microcystin nor methylHg (meHg) are currently measured or treated-for in village treatment systems. Important terrestrial subsistence species, such as caribou, moose, and muskox all use surface water sources in the ice-free season, and may be at risk from water-borne bacterially-methylated Hg⁽¹⁾.

Warmer winters may have favored survival of land and sea mammals with zoonotic infections, with resulting zoonotic antibody seroprevalence in these animals similar to those found in the southern Bering Sea and more southern regions of Alaska. Zoonotic infectious pathogens not previously known to exist in the Chukchi Sea and Arctic Slope region may have extended their range⁽³⁾. The same pathogen movement is also possible as more southern land mammals, such as the beaver and muskrat, which have moved north, may have brought pathogens such as giardiasis and *Franciscella tularensis*, the bacteria which causes tularemia⁽⁴⁾.

Due to an increase in coal-fired power plants in Asia, more oceanic and atmospheric Hg is being transported to and deposited in Alaska, where Hg is methylated by bacteria and becomes methylHg (meHg), the most toxic form of Hg⁽⁵⁾. All forms of Hg and anthropogenic

organohalogen compounds (OH) bioaccumulate in the Chukchi and Beaufort Sea mammals, but little sampling has been reported, compared to the numbers of sea mammals harvested in this region annually.

Climate warming in the circumpolar north has resulted in the following changes: Alaska's mean annual air temperature has increased approximately 3 degrees C over the past 4 decades; the permafrost temperature has increased in some areas, and the active layer above the permafrost has increased in depth; ice-free seasons are longer and warmer⁽⁶⁾; there has been extension of the range of southern Bering Sea marine species and southern Alaska terrestrial species further north⁽⁷⁾. Changes are taking place in ocean and atmospheric transport of anthropogenic contaminants, and uptake of organohalogen contaminants (OH) and mercury (Hg) into food webs⁽¹⁾. Ecosystem changes in the Bering Sea have been associated with northward movement of food-borne and water-borne zoonotic pathogens and some of these have not previously been known to infect sea mammals in the Bering and Chukchi Sea⁽³⁾.

Rural Alaska Natives (AN) are, by virtue of isolated location, culture, and economic status, certainly one of the most subsistence-dependent population in the US⁽⁸⁾. Approximately 50% of the AN population lives in very small settlements of 100-500 people each, in remote sections of rural Alaska with no road access. They depend on very expensive air transport or water transport in the ice-free season, for any goods not available from the environment. Most of these communities could not afford to replace the subsistence wildlife contribution to their diet with western foods, and thus traditional subsistence foods are a central part of the culture, and are economically critical to the existence (sustainability) of the village⁽⁸⁾.

Advances in medical care for Alaska Natives have decreased infant mortality, increased life expectancy, and improved cancer survival. More chronic illnesses are appearing, such as

diabetes and asthma, and more Alaska Natives are being treated with medications that can impair immune response ⁽⁹⁾.

The northern marine subsistence diet has well-documented population health benefits ^(10, 11), as well as great cultural importance ⁽⁸⁾.

A simple, inexpensive method of testing the blood of subsistence-killed animals for exposure to zoonotic pathogens and contaminants would allow communities to adapt their harvest practices in a way that would reduce exposure to these climate-sensitive environmental risks.

Methods

The ANTHC Climate Change Health Assessment (CCHA)

The initial focus of the RAMP development required the creation of a community environmental health impact assessment tool that could be used to qualitatively describe the impact of climate mediated change on an individual village, recognizing that in different Arctic regions, with differing climates and ecosystems, different impacts could predominate. Over a 3 year period, the ANTHC Climate Change Health Assessment (CCHA) tool was developed and refined, and has now been applied, by request, to three villages in the Arctic LCC region, as well as communities in the Bering Strait region, in the Norton Sound region, and in the Southwest region of Alaska.

The CCHA is a four step process, fully described in a recent paper ⁽¹²⁾, briefly summarized below.

1. Scoping: to describe local conditions and engage stakeholders;
2. Surveying: to collect descriptive and quantitative data;
3. Analysis: to evaluate the data; and

4. Planning: to communicate findings and explore appropriate actions, including adaptation strategies, and where possible, mitigation measures, with community members;

These actions also include consideration of whether the community wishes to begin a monitoring program. This includes development of monitoring metrics, and steps in the creation of an adaptation strategy to reduce risk, especially for community members with special characteristics (pregnancy, infants, people taking immunosuppressive medications) who may be at increased risk from the identified threat.

The CCHA process identified many environmental threats, including those related to the safety issues surrounding harvesting, handling, preparing and consuming of traditional foods; safety and availability of the community source water and other traditional water supplies; thawing of permafrost and resultant threats to village sites, buildings, and critical infrastructure such as treatment plants, sewage lagoons, boardwalks, and schools.

The CCHA focuses on local observations and traditional seasonal time scales and synthesizing climate and health casual chains. Its success depends on a broadly participatory framework of community members, various state and federal agencies, and university researchers, and by necessity combines Indigenous and Western knowledge systems.

The findings of the Selawik CCHA, one of the deliverables in the ALCC grant, have been presented to the village, and can be viewed at this link:

<http://www.anthc.org/chs/ces/climate/bbs/climateandhealthreports.cfm>

ANTHC Local Environmental Observer (LEO) network

The initial response to the need to improve the awareness and community engagement with the many impacts of climate warming in rural Alaska has been the development and deployment of

two mechanisms. The first mechanism is a village-based local environmental observer (LEO) network, established by ANTHC in 2011 www.ANTHC.org/chs/ces/climate/leo/. Village residents, all of whom are environmental program employees, will be trained in the filter paper sampling methodology described in this subsection, such that they can subsequently train village hunters in the method. They will perform blood sample collection from subsistence-hunted animals. Training will also include labeling, processing and shipping of the blood samples. A supporting web-based reporting and educational system has already been developed and put in place at: <http://www.anthc.org/chs/ces/climate/leo/index.cfm> to support web-based education, and serve as a location for webinars on environmental topics. This website also provides a place where LEOs can post observations, share experiences with other LEOs and with consultants from ANTHC, state and federal agencies and universities. The LEO system depends on both traditional ecological knowledge, and western science, which are merged in the observations posted by the LEOs, and in the responses to the observations posted by western science consultants. It has also allowed early recognition of serious ecosystem issues, as exemplified by earliest Arctic slope hunter observations of sick and dead seals with skin lesions, culminating in the 2012-2013 NOAA Seal Mortality Event (UME)<http://nmfs.noaa.gov/pr/health/mmume/>. Subsequent posts by LEOs in the Chukchi and Bering Strait region provided data on the widespread distribution of this condition, and its subsequent disappearance. The LEO network provides a model for engaging tribal and other communities to apply local and traditional knowledge, perform surveillance, and connect with western scientific technical experts, and thus provide the optimal input to the community for effective adaptation. The LEO system has spread rapidly within Alaska, with over 150 LEOs in more than 100 Alaska villages, and new LEO communities in western Arctic Canada and British Columbia.

Filter Paper Blood Sampling of Subsistence Animals

The second mechanism, filter paper blood sampling to improve community monitoring for zoonotics and contaminants in subsistence harvested animals, has been developed as part of the objectives of this grant, and is just beginning to be fully deployed in rural Alaska. The method utilizes an innovative approach using filter paper strips to absorb blood for sampling. It was developed and described for zoonotic pathogen antibody detection by the University of Alberta to sample blood from animals harvested by Inuit hunters⁽¹³⁾. ANTHC used funding support from this grant to contract with the University of Alberta to train the staff at the University of Alaska Fairbanks Wildlife Toxicology Laboratory (WTL) in the technique. The WTL has become proficient in eluting antibody-containing serum from the blood-soaked strips, and has completed quality assurance development for antibody testing. The testing of subsistence harvested specimens began in fall, 2013, and will continue through the next 4 years, with emphasis on caribou and sea mammal samples gathered at sites with historically high harvest rates. The WTL has used this grant funding to further develop the filter paper methodology to facilitate analysis of blood total Hg levels from the strips⁽¹⁴⁾. This is, to our knowledge, the first time this has been done. The filter paper specimens from Alaska land and sea mammals will undergo preliminary processing at the WTL to have antibody-containing serum eluted from the blood-soaked strip, and the serum specimens sent to selected laboratories and State of Alaska laboratory consultants for determination of antibodies to *Toxoplasma gondii*, *Coxiella burnetti*, brucella, and other pathogenic zoonotic organisms. Sea mammal filter paper blood strips will be analyzed for total blood Hg levels at the WTL. Use of blood-on-filter paper methods are developed, but field implementation remains limited due to the time required to create test protocols, and conduct and complete quality assurance testing with different antibody levels, and

different pathogens. This grant expanded the training and development of instructional materials for this methodology, and provided for increased distribution of test kits in the Bering Strait region. This method will be utilized to gather 200 caribou samples and 100 sea mammal samples from the Arctic LCC region in the fall of 2014 and spring of 2015 and yearly thereafter.

Development of filter paper blood specimens for organohalogen analysis

The use of blood-on-filter paper samples to measure levels of lipid soluble OHs, has not been used in animals, but was initially pilot tested successfully in humans by the Center for Disease Control (CDC)⁽¹⁵⁾. ANTHC has partnered with the WTL to pursue method development for the OH analysis using filter paper blood strips, and this should be completed by June, 2014. The successful use in humans suggests that the method should be applicable in subsistence animal blood, as well. OHs do not generally accumulate in Arctic terrestrial herbivores, so they will not be tested for OHs. However, herbivores are a possible source of Hg, and are a potential source of zoonoses, especially brucella, toxoplasma, and *coxiella burnetti*, the agent of the disease Q fever⁽⁷⁾. We will also sample blood from sea mammals harvested in the Bering Straits, Chukchi Sea, and Beaufort Sea and test for Hg, OH and the same three zoonotic antibodies described previously. The target is 200 land mammals and 100 sea mammals each year as long as funding sources are available. See the LEO website for a link to the short video of the use of the filter paper test strips: <http://www.youtube.com/watch?v=BxYU3y5Vk9k>

RAMP Grant Program Results

1. The Selawik CCHA can be viewed at:

<http://www.anthc.org/chs/ces/climate/bbs/climateandhealthreports.cfm>

2. The filter paper blood test development by the UAF WTL is described in the attached peer-reviewed paper, which may be cited as:

Hansen C., Hueffer K., Gulland F., Wells R., Balmer B., Castellini M., O'Hara T. 2014. Use of cellulose filter paper to quantify whole blood mercury in two marine mammals: validation study. *Jour. Wildlife Dis.* 50:271-278.

Conclusions

The communities of the Bering Strait, Chukchi and Beaufort Sea coastal regions are very interested in, and willing to participate in, village-based environmental monitoring. Interest is also present in Arctic Canada. Communities in Arctic Canada are using filter paper sampling to monitor zoonotic exposure in caribou. In Alaska, we have begun filter paper sampling training for village hunter's with field sampling on a large scale to begin in the 2014 caribou season.

Blood-on-filter paper sampling of large sea mammals harvested in the Bering Straits region will commence in spring, 2015. Distribution of sampling kits and training materials continues to be requested by villages in Arctic Alaska. The community LEOs are very interested in coordinating these efforts, and are a key part of the effort.

The CCHAs have been enthusiastically received by rural AM communities, with additional CCHAs requested by other communities. These assessments have proven to be an ideal way to provide villagers with an introduction to RAMP as a way to evaluate emerging climate-driven threats, and develop adaptation strategies that should allow for the continued use of traditional diets.

The use of filter paper blood samples can likely be extended to the analysis of lipophilic organic contaminants, and methods for extracting these contaminants are currently under development.

Wildlife Health Applications

Hunter-provided filter paper blood samples from subsistence harvested animals taken in circumpolar regions would enable international assessment of regional seroprevalence of zoonotic and wildlife-specific pathogens. Over time, this would enable correlation with climate and oceanographic information to develop predictive models of pathogen range and range changes, informing region-specific management plans. The same samples could provide data on metals (Hg, Pb, Cd). Regions of higher concentrations could indicate either natural sources, or areas of concentrated deposition, and furnish useful information for regional and national environmental agencies.

Human Health Applications

The basic community environmental assessment tools used in the RAMP, and subsequent environmental threat identification, could be applied in any region in the circumpolar north, with development of a community or regional adaptation and monitoring plan to reduce risk and monitor the trends in established threats, and detect emerging threats.

Filter paper blood spots are widely used in population screening in human public health applications, particularly in screening for newborn metabolic diseases. The use of this technique of sampling could just as easily be used for screening rural human populations for antibodies to zoonotic pathogens. This information would be useful to regional public health organizations in the following ways: the data would provide the level of exposure to pathogens, and could indicate groups at greatest risk of exposure; the data could be used to raise awareness in hunters and consumers that precautions are warranted in game handling and field dressing, as well as storage, preservation, and cooking practices; consumers and medical care providers could be educated in the symptoms of active infection, so identification and proper diagnostic tests and

appropriate treatment could be provided; identification of vulnerable subsets of residents could be identified so that providers and residents could be made aware of which demographic subsets of people who are more likely to develop symptomatic serious infections resulting from zoonotic pathogen exposure. Examples are elders, infants, pregnant women, people with diabetes, cancer survivors (especially those on chemotherapy), and people on immunosuppressive drugs for other conditions, such as asthma and autoimmune disorders.

Future Plans

We plan to offer training to communities around the circumpolar north in the use of filter paper sampling for antibody detection and Hg measurements on subsistence-killed animals, starting in Canada, and extending to Greenland and Europe, with the aid of colleagues in the Arctic Council Human Health Assessment Group, and the Human Health Expert Group. Members of these working groups have extensive contacts with rural Arctic residents in their respective countries. We also plan to continue development of methodology allowing use of filter paper samples for assay of organohalogen contaminants, with the aid of colleagues at the University of Alaska, and laboratory consultants.

ANTHC wishes to thank the Arctic Landscape Conservation Cooperative for the support of this project. We also wish to acknowledge the other state and federal agencies, as well as the residents and regional officials of the Arctic slope and northwest Alaska, for their generous support, and willingness to make the critical contribution of their time and traditional knowledge, without which the RAMP would not be possible.

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USE OF CELLULOSE FILTER PAPER TO QUANTIFY WHOLE-BLOOD MERCURY IN TWO MARINE MAMMALS: VALIDATION STUDY

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ABSTRACT: Whole blood (WB) is commonly used to assess mercury (Hg) exposure in mammals, but handling and shipping samples collected in remote areas can be difficult. We describe and validate use of cellulose filter paper (FP) for quantifying WB total Hg concentration. Advantec Nobuto[®] FP was soaked with bottlenose dolphin (*Tursiops truncatus*) or harbor seal (*Phoca vitulina*) WB (collected between March and July 2012), then air dried. Untreated blood-soaked FPs were analyzed or were eluted with phosphate-buffered saline (PBS) and the eluate and PBS-treated FP Hg concentrations were determined. Total Hg from dried blood-soaked FPs, postelution FPs, and PBS-based eluate were compared with total Hg concentrations from WB. Recovery (on a concentration basis) for soaked FP relative to WB was 0.89 ± 0.15 , for postelution FP was 0.86 ± 0.13 , and for eluate (with a correction factor applied) was 0.96 ± 0.23 . Least-squares linear regressions were fit for soaked papers ($y = 1.15x$, $R^2 = 0.97$), postelution FPs ($y = 1.22x$, $R^2 = 0.95$), and for eluate with a correction factor applied ($y = 0.91x + 0.03$, $R^2 = 0.97$) as compared with WB. These data show that FP technology can have a valuable role in monitoring blood Hg concentrations in wildlife populations and FPs have the advantage of being easy to use, store, and transport as compared with WB.

Key words: Biomonitoring, marine mammal, Nobuto filter paper, total mercury, wildlife.

INTRODUCTION

Mercury (Hg) is a nonessential element that occurs naturally in the environment. Mercury is released into the atmosphere via events such as volcanic eruptions and forest fires. Since the industrial revolution, anthropogenic releases of Hg into the environment have increased, mostly through the burning of fossil fuels and via the mining industry, and may occur at concentrations of concern to health in some biota (e.g., Dietz et al. 2009, 2013). Following deposition of atmospheric Hg into marine and freshwater systems, microbial activity (largely sulfate-reducing bacteria) can transform Hg to the highly bioavailable and toxic monomethylmercury (MeHg⁺) (Fitzgerald et al. 2007; Parks et al. 2013). Monomethylmercury can bioaccumulate and biomagnify with trophic levels (Coelho et al. 2013), reaching

particularly high levels in numerous fish species and piscivores (Castoldi et al. 2001; Lemes et al. 2011; Castellini et al. 2012).

After ingestion, MeHg⁺ is absorbed via intestinal epithelium passively and via active uptake (Leaner and Mason 2002), and is nearly completely absorbed. Crossing the intestinal epithelium, MeHg⁺ enters the blood where 99% binds to thiol groups; the remaining 1% is transported to organs via binding to diffusible low-molecular-weight thiols (Rooney 2007). Hence blood is the route of exposure (and distribution) for most target organs (i.e., the central nervous system) and is a reliable indicator of recent MeHg⁺ exposure (Risher and Amler 2005).

A key target organ for MeHg⁺ toxicity is the central nervous system as MeHg⁺ crosses the blood–brain barrier via an amino acid transporter and accumulates in nervous tissue (Kerper et al. 1992; Caito

et al. 2013). Clinical signs of acute toxicity include proprioceptive deficits, abnormal postures, blindness, anorexia, coma, and death (Ekino et al. 2007). High levels of MeHg^+ have been shown to impair components of the nervous system (Basu et al. 2006, 2007b). There is concern that, particularly in fish-eating wildlife, chronic exposure to MeHg^+ can result in poor reproductive success (Basu et al. 2007a). There is also concern that Hg levels in wildlife and in humans that subsist on wildlife (particularly in higher latitudes) may be reaching concentrations that can have impacts on behavior and health (e.g., Castoldi et al. 2001; Basu et al. 2009), especially for the fetus and neonate (Castellini et al. 2012; Rea et al. 2013).

Whole blood (WB) is commonly used to assess Hg exposure (Brookens et al. 2007; Knott et al. 2011). Blood is relatively easy to access (relative to target tissues such as the kidney and nervous system), is commonly collected by biologists, veterinarians, and others who work with wildlife in the field, and is a good tissue for determining Hg status in wild animal populations. Hair is easily accessible and used for monitoring Hg status in wildlife and is more useful for long-term (chronic) mercury assessment, as hair Hg concentration represents the average concentration of Hg in circulating blood (Budtz-Jorgensen et al. 2004).

There are long-term mercury monitoring programs in place for wildlife, particularly fish (US EPA 2012), and monitoring sometimes follows contamination events (Alvarez et al. 2013). Monitoring programs for humans exist as well (Alaska Epidemiology Bulletin 2013; ANTHC 2013). However, blood is less commonly used for biomonitoring due to relative difficulty (compared with hair) with collection, storage, and transport. Collection in the field can be problematic, especially in remote locations with limited processing and preservation capabilities. The development of a blood-sampling regime that can be easily used in the field by scientists,

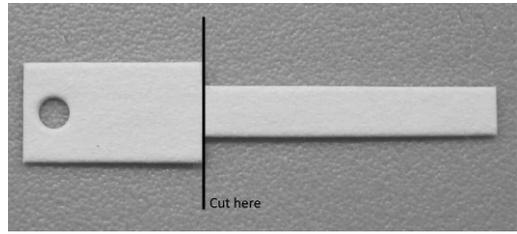


FIGURE 1. Single Nobuto filter paper strip. Black line indicates where strip should be cut after soaking in blood and drying, and before elution (Fig. 2).

hunters, fishermen, or other trained people would facilitate clinical, research, and biomonitoring efforts. We describe the use of cellulose filter paper (FP) for collection of blood in the field and subsequent analysis of total Hg concentration in various FP matrices in comparison with WB collected in standard blood collection tubes.

MATERIALS AND METHODS

Filter paper and samples

Nobuto[®] cellulose FP (Advantec, Dublin, California, USA) was purchased from Cole-Parmer (Vernon Hills, Illinois, USA) and was used for all investigations (Fig. 1). Filter paper was used singly or fashioned into combs of five to six papers for use in the field (Curry et al. 2011). Whole-blood samples were collected between March and July 2012 from wild harbor seals (*Phoca vitulina*) brought to The Marine Mammal Center (Sausalito, California USA, Marine Mammal Protection Act permit 932-1905/MA-009526) for rehabilitation and from long-term resident bottlenose dolphins (*Tursiops truncatus*) live-captured, sampled, and released after health assessments in Sarasota Bay, Florida during May and July 2012 by staff from the Chicago Zoological Society (Wells et al. 2004; National Marine Fisheries Service Scientific Research Permit 15543, Institutional Animal Care and Use Committee 11-09-RW1). Blood samples were collected into BD (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) Vacutainers[™] containing K_2 -ethylenediaminetetra-acetic acid as an anticoagulant. The narrow absorbing ends of 10–12 FP were soaked in WB (approximately 100 μL /strip) after collection and FP was air-dried overnight. The fluid sample of WB was stored frozen (-20 C). For each animal, dried FP

samples were shipped overnight at room temperature in a sealed plastic bag with paper towels layered between each sample and blood samples were shipped accompanied by freezer packs to the Wildlife Toxicology Laboratory at the University of Alaska Fairbanks, Fairbanks, Alaska, USA.

Sample preparation

Before chemical analysis, control ($n=10$, no blood) and blood-soaked FP were freeze-dried for 48 hr in a Labconco FreeZone 6 Plus freeze dryer (Kansas City, Missouri, USA). The narrow absorbing ends of FP_C (control) and FP_{WB} (soaked, WB) were cut (using a disposable razor blade) at the junction of the narrow and wide ends (Fig. 1) and weighed to determine the dry mass of blood on each paper (mass WB = mass FP_{WB} - average mass FP_C). All 60 FP_C and three FP_{WB} from each individual animal sample set were analyzed directly for total mercury concentration ([THg]). The [THg] was calculated on the basis of the mass of mercury (nanograms) and mass of blood (~100 mg) on each strip. Three more FP_{WB} from each individual animal sample set were separately eluted according to the protocol developed by Curry et al. (2011). Each FP_{WB} was cut into five to seven pieces into a 2-mL preweighed cryogenic tube (Thermo Scientific, Waltham, Massachusetts, USA) using stainless steel iris scissors. Each strip was then covered with 400 μ L of phosphate-buffered saline (PBS; Gibco, Carlsbad, California, USA) with 1% penicillin-streptomycin (Gibco). Each cryogenic tube was agitated to ensure that FPs were soaked, and were eluted overnight (16 hr) at 4 C.

After 16 hr, approximately 200 μ L of eluate (E) were removed from each cryovial using a micropipettor. Eluate was transferred to a 1.5-mL microcentrifuge tube (Fisher Scientific, Waltham, Massachusetts, USA) and held at -50 C until analysis. Postelution FPs (including ~200 μ L of remaining eluting buffer) were again freeze dried for 48 hr. After drying, each cryovial (containing postelution FP pieces) was weighed to determine the final weight of the postelution paper (FP_E).

Mercury analysis

All samples (WB, FP_C, FP_{WB}, FP_E, and E) were analyzed for [THg] on a Milestone DMA-80 direct mercury analyzer (Milestone Inc., Shelton, Connecticut, USA; US EPA method 7473) using a 16-point calibration curve from 0.25 ng to 400 ng, similar to Knott et al. (2011). Samples were analyzed in triplicate when possible (i.e., when there were enough FPs for each sample). Single FPs (for

FP_E a single FP included five to seven cut pieces) were analyzed in nickel sample boats and WB (~100 μ L) and eluates (100 μ L) were analyzed in quartz sample boats. The detection limit using this method was 5 ng/g for 100 μ L of blood or eluate and 2.5 ng/g for 200 μ L of eluate.

Quality control included a 10-ng (1 μ g/g) liquid calibration standard (Perkin Elmer, Waltham, Massachusetts, USA, item N9300133), and DORM-3 (National Research Council Canada, Ottawa, Ontario, Canada) and DOLT-4 (National Research Council Canada) certified standards analyzed in triplicate in each DMA80 run. Recoveries were $94.6 \pm 5.2\%$ (10 ng), $102.2 \pm 4.4\%$ (DORM-3, reference range 0.382 ± 0.060 mg/kg), and $100.1 \pm 6.8\%$ (DOLT-4, reference range 2.58 ± 0.22 mg/kg).

Calculations and statistics

Data were managed in Microsoft Excel, and statistics were performed using program R (R Development Core Team 2013). Least-squares linear regressions were fit to FP_{WB}, FP_E, and E compared with WB. Confidence intervals (95%) for slopes were constructed, and slopes were compared with a test value of 1 using R. Student's paired *t*-tests were used to compare [THg] means of FP_{WB}, FP_E, and E with WB.

Whole-blood data were converted to a dry-weight basis using the proportion of dry matter in WB. For some calculations and statistics wet weight concentrations are reported; for others, dry weight concentrations. To determine the dry weight of blood from each species, 100 μ L of WB were weighed, freeze-dried for 48 hr, and reweighed. The dry blood weights were $24.9 \pm 1.8\%$ for harbor seals and $20.5 \pm 0.7\%$ for bottlenose dolphins.

A correction factor was applied to eluate samples to estimate the original WB (wet) [THg] (Fig. 2). The elution process involves adding 400 μ L of PBS (~0.400 g) to strips (FP_{WB}) containing dried components (0.2–0.25 g) from approximately 100 μ L (~0.100 g) of blood. Therefore a correction factor (CF) was estimated for each sample as follows:

$$CF = \text{mass of E} / \text{mass of WB}_{\text{wet}} = (0.400 \text{ g} + \text{weight [in g] of dry blood on FP}_{\text{WB}}) / 0.100 \text{ g}.$$

This correction factor was then applied to eluate [THg]:

$$E_{\text{CF}} = E \times CF \approx \text{WB (wet)}$$

This correction factor result was compared with the original WB (wet) [THg].

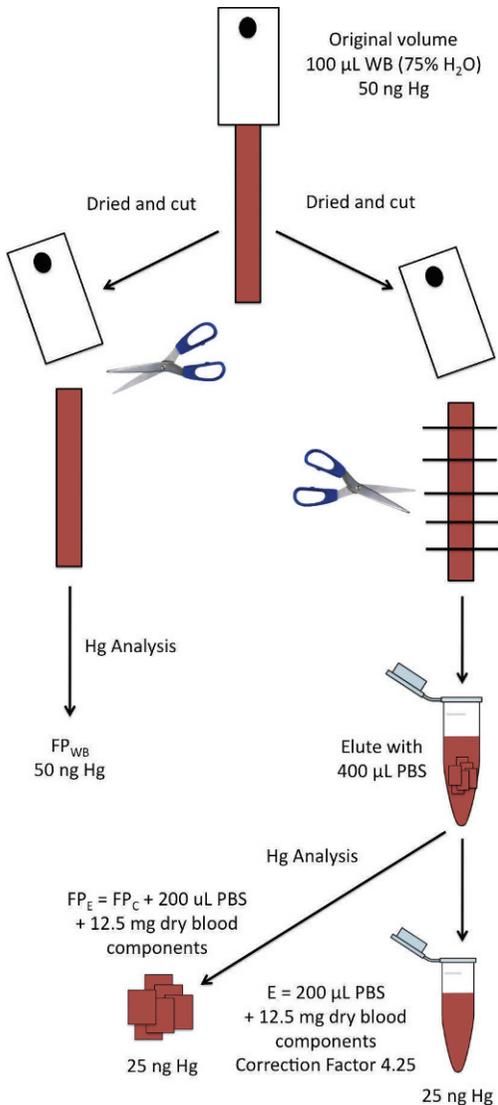


FIGURE 2. A conceptual model of the elution process. Each filter paper is soaked in approximately 100 µL of whole blood (WB) (original volume). After drying, approximately 20–25 mg of dry blood products remain on filter paper (FP_{WB}). These FP_{WB} can be analyzed for total mercury concentration [THg] directly, or eluted as follows. The dry blood products on FP_{WB} are eluted in 400 µL of phosphate-buffered saline (PBS), 200 µL are collected as eluate (the remaining 200 µL remain soaked into the filter paper [FP_E]). FP_E or the eluate (E) can then be analyzed for [THg].

RESULTS

The average weight of the narrow part (Fig. 1) of FP_C is 0.0466 ± 0.002 g ($n = 10$).

Single control FPs not soaked with blood were below the detection limit of the DMA-80 (0.5 ng/FP, $n = 10$). Mean [THg] values (on a concentration basis) for WB, FP_{WB}, FP_E, and E are summarized in Table 1. FP_{WB}, FP_E, and E [THg] relative to [THg] in WB in matched samples are summarized in Figure 3. For dolphins, the relative proportion of [THg] in FP_{WB} and FP_E compared with WB is 0.87 ± 0.08 and 0.82 ± 0.13 , respectively. For harbor seals, the relative proportion of [THg] in FP_{WB} and FP_E compared with WB was more variable at 0.95 ± 0.42 and 0.92 ± 0.32 , respectively. The mean difference between the proportion of [THg] FP_{WB} compared with WB is 0.04 ($P < 0.001$), between FP_E and WB is 0.05 ($P < 0.001$), and there is no mean significant difference between E_D WB ($P = 0.4$; paired t -tests).

Figure 4 shows [THg] WB regressed on FP_{WB}, FP_E, and E values. Data for WB, FP_{WB}, FP_E, and E are presented on a wet-weight basis. The R^2 for blood-soaked FP is 0.97, for postelution FP is 0.95, and for eluate (with correction factor applied) is 0.97. A 95% confidence interval for the slope is 1.12–1.19 for WB regressed on FP_{WB}, 1.18–1.32 for WB regressed on FP_E, and 0.89–0.97 for WB regressed on E. Tests for each slope (H_0 : slope=1 or $y=x$) indicates $P < 0.01$ for each regression (Fig. 4).

DISCUSSION

We used blood-soaked FP samples to assess mercury concentrations in the blood of bottlenose dolphins and harbor seals. The values for WB total mercury for bottlenose dolphins and harbor seals from our study populations (Table 1) are within the ranges previously reported (Brookens et al. 2007; Woshner et al. 2008).

Advantec Nobuto FP strips are uniform in size and weight (0.0446 ± 0.002 g), and their [THg] is below the detection limit of a DMA80 (< 0.5 ng). Our data support that cellulose FP soaked in WB and air-dried is an accurate and reproducible way

TABLE 1. Mean, range, SD, and sample number (*n*) for total mercury concentration [THg] in bottlenose dolphin (*Tursiops truncatus*, *n*=25) and harbor seal (*Phoca vitulina*, *n*=34) whole blood (WB), filter paper soaked in whole blood (FP_{WB}), postelution filter paper (FP_E), and eluate (E) samples.

Species	WB (µg/g)		FP _{WB} (µg/g)		FP _E (µg/g)		E (µg/g)	
	Wet ^a	Dry ^b	Wet ^b	Dry ^a	Wet ^b	Dry ^a	Wet ^a	CF ^c Applied ^b
Bottlenose dolphin								
Mean	0.48	2.39	0.41	2.06	0.39	1.97	0.12	0.50
SD	0.33	1.66	0.28	1.42	0.27	1.42	0.08	0.35
Range	0.12–1.34	0.61–6.71	0.09–1.15	0.47–5.75	0.09–0.97	0.45–5.67	0.03–0.35	0.13–1.51
Harbor seal								
Mean	0.16	0.64	0.14	0.56	0.13	0.55	0.03	0.13
SD	0.11	0.42	0.10	0.38	0.09	0.36	0.02	0.09
Range	0.03–0.45	0.12–1.78	0.03–0.42	0.12–1.67	0.04–0.41	0.14–1.66	0.01–0.11	0.03–0.46

^a Measured.
^b Calculated.
^c CF=correction factor.

to quantify WB [THg] for some mammals. Overall recoveries on a concentration basis are very high, ranging from 82 to 95%, when compared with WB concentration for FP_{WB}, FP_E, and E_{CF} (Fig. 3). Additionally, with *R*² values of 0.97, 0.95, and 0.97 respectively for FP_{WB}, FP_E, and E_{CF} (Fig. 4), WB mercury concentration can be easily estimated from dried or

eluted samples, provided [THg] is high enough to be detected.

This technique promises to be valuable to scientists, wildlife managers, veterinarians, and others needing a simple, inexpensive, and highly effective method for collecting blood samples for mercury analysis in combination with other assays. Perhaps even more important, these FPs could be distributed to hunters and used in the field to increase the scope of wildlife-monitoring programs. Programs aimed at developing community-based wildlife health-monitoring programs exist (Brook et al. 2009; ANHSC 2013), and distribution of FP sample kits (including instructions and prepaid shipping labels) through outlets like these would benefit mercury and other disease/health-monitoring efforts around the globe (Curry et al. 2011).

Our findings demonstrate that mercury in blood elutes readily, and our methods allow half of the eluate and roughly half of the mercury to remain with the postelution filter paper (Fig. 4). We also show that Hg-associated dry components of blood likely distribute in a similar way by using a correction factor that demonstrated results with a strong correlation to WB [THg]. Because mercury is bound to sulfhydryl groups on hemoglobin molecules

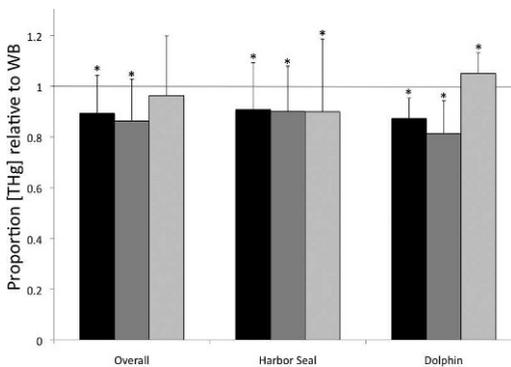


FIGURE 3. Proportion of total mercury concentration [THg] (µg/g) in filter paper with whole blood (black bars), eluted filter paper (medium gray bars), and eluate (light gray) relative to whole blood (WB=1) for bottlenose dolphin (*Tursiops truncatus*, *n*=25) and harbor seal (*Phoca vitulina*, *n*=34) samples. Error bars indicate 1 SD from the mean. * Indicates significant difference in means of paired samples when compared with WB as gold standard (*P*<0.05).

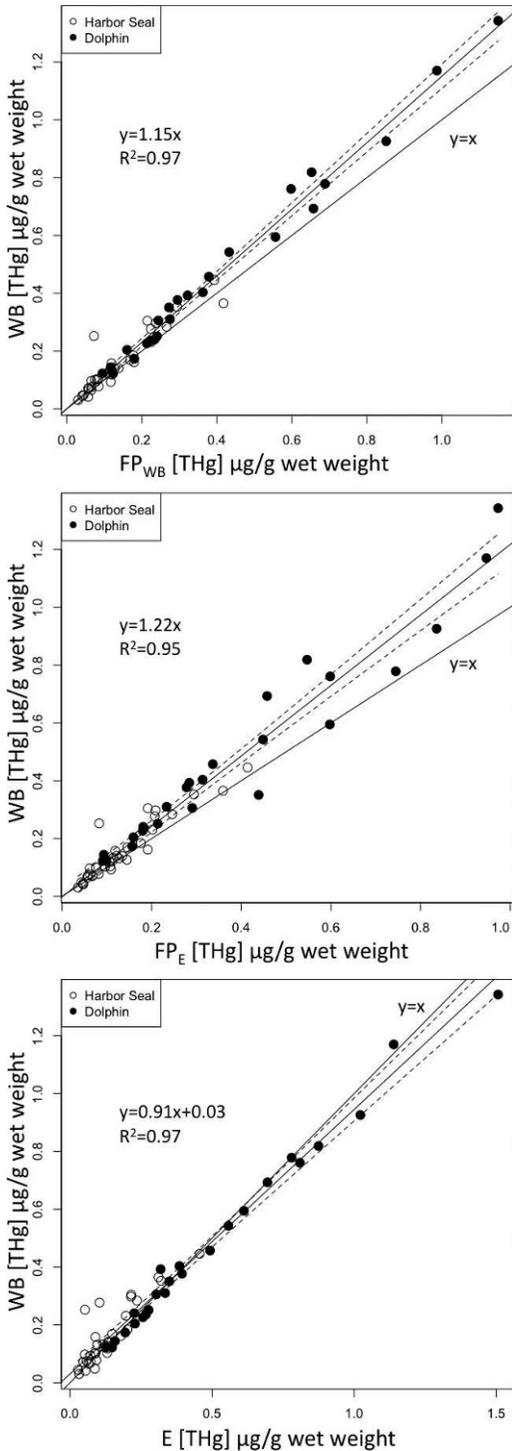


FIGURE 4. Linear regressions of whole blood (WB) on filter paper (FP) with WB (FP_{WB}), FP with eluate (FP_{E}), and eluate only (E) (with dilution factor applied). All slopes are not equal to 1. Dashed lines are a 95% confidence band for the slope. A line of unity ($y=x$) is shown in each panel.

(Weed et al. 1962), we hypothesize that the hemoglobin is following this same pattern and is moving into the eluate, and half of that remains on the FP_{E} with the residual 200 μL of buffer. On the basis of this we have developed a conceptual model of the elution process describing the utility of predicting WB [THg] directly using blood-soaked FP_{WB} and indirect methods that use certain postelution products (FP_{E} , E; Fig. 2).

Although blood is not as easy to collect as hair, FP technology facilitates blood collection and makes it easier to store and ship air-dried blood. Hair provides a long-term picture of mercury status (Budtz-Jorgensen et al. 2004), whereas blood represents short-term exposure, and is the route of exposure for target organs (the central nervous system and kidneys). The combination of dried FP and hair samples, both of which can be stored at room temperature and shipped under ambient conditions, will allow wildlife scientists to obtain a more complete picture of the mercury status in populations of interest.

The designed use of these FPs is for protein (antibody) preservation for antibody detection (serology). We have shown the added advantage of being able to use FP_{WB} , FP_{E} , or E for quantifying mercury in WB. Previous studies have used FP eluate to validate serologic use in wildlife populations (Curry et al. 2011). We emphasize the excellent correlations between [THg] in WB and both FP_{E} and E (Fig. 4). Thus, one can utilize the FP eluate as intended for serology, and use any remaining FP_{WB} or FP_{E} to quantify mercury. This type of use could be a significant advantage if the available blood volume is limited, either in small species or in situations where hunters or wildlife professionals are unwilling or unable to obtain large quantities of blood.

One unknown factor pertains to the shelf life of these samples. All of our analyses were conducted within 8 mo of collecting samples on FP. It would be

important to see if similar results would be obtained with long-term storage. However, we do not anticipate volatilization or degradation to be significant for [THg] measures as compared with more vulnerable components such as antibodies.

This FP technique promises to be broadly applicable wherever field sampling of WB for [THg] is needed. The strips can be air dried, do not need to be refrigerated, and theoretically have a long, stable shelf life once samples are collected. This method will be particularly useful in monitoring [THg] in subsistence foods in remote Alaskan communities, where Alaska native peoples often subsist on fish-eating marine mammals. Application of this technology to human fish-consumer blood sampling, in conjunction with hair-monitoring programs, should also be considered.

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