

Sulfide Mining and Human Health in Minnesota

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Sulfide mining (specifically copper-nickel sulfide mining) represents a significant departure from Minnesota's iron mining tradition. Sulfide mining can produce acid waste and sulfates that mobilize the release of heavy metals into the environment. These metals include known neurotoxins such as lead and mercury. Mining activities also create airborne fibers and pollutants that can contribute to increased morbidity. The short- and long-term effects of exposure to these substances on human health should be considered in present and future sulfide mining proposals. In addition, Minnesota physicians need to understand the potential adverse mental and physical health effects of sulfide mining on mine workers and residents of communities near mining operations.

The Duluth Complex is a geological formation that contains deposits of copper, nickel and palladium group metals. It is located at the eastern end of the Mesabi Iron Range in northeastern Minnesota.¹ PolyMet Mining plans to build an open-pit mine in the northeastern part of the state to recover those valuable metals. Other mining companies are also exploring mineral deposits and preparing proposals for extracting them.

During the past three years, multiple organizations representing health care professionals have voiced concern about the potential effects of copper-nickel mining on human health. The Minnesota Medical Association, Minnesota Public Health Association, Minnesota Nurses Association and Minnesota Academy of Family Physicians have each endorsed deeper inquiry

FIGURE 1

Metal sulfide oxidation sequence using pyrite as an example

Pyrite (FeS_2) is exposed to air (O_2) and water (H_2O)

A sequence of reactions occur creating sulfate, ferric hydroxide and hydrogen ions.

Additional chemical reactions occur involving pyrite, iron sulfate and water, resulting in the release of sulfuric acid.

Sulfuric acid promotes release of other metals from rock and causes harm to aquatic ecosystems.

into the potential health effects of sulfide mining, and specifically of copper-nickel mining. Although the majority of debates about sulfide mining in our state have been framed as “environment versus jobs,” the impact on human health needs to be a part of these discussions.

Sulfide mining has significant potential for the release of toxic chemicals into the environment. These include a number of chemicals identified by the World Health Organization as being of major public health concern: arsenic, asbestos, cadmium, lead and mercury.² Given this ominous list, and the possible synergistic effects of co-exposure to more than one of these chemicals, it is important that physicians understand why concerns are being raised about this type of mining. ➡

Source: Jacobs JA, Lehr JH, Testa SM. Acid Mine Drainage, Rock Drainage and Acid Sulfate Soils: Causes, Assessment, Prediction, Prevention and Remediation. Hoboken, NJ: John Wiley & Sons; 2014

How Acid Mine Drainage is Generated

Sulfide mining differs significantly from iron ore (taconite or ferrous) mining because it has the potential to generate acidic pH. Copper and nickel typically are bound to sulfur in rock. Because of this sulfur bond, they are described as sulfide minerals.³ The chief iron-bearing minerals in iron mining are iron oxides and iron carbonate,⁴ neither of which are sulfide minerals. Typical iron ore in Minnesota is relatively poor in sulfide minerals and contains minerals that actually buffer acid generation. Minnesota has not experienced large-scale release of toxic metals from iron mine waste into the environment.

However, both iron and sulfide mining operations do involve the excavation of millions of tons of rock in order to acquire a fractional amount of desired product. The ore is then processed to yield the desired metal. The surface mine site as well as mining wastes (overlying material, waste rock and “tailings”—fine-grained materials left over after the metals of interest are extracted) are exposed to moisture and atmospheric oxygen. When the sulfide mineral ore and wastes come into contact with air and water, chemical reactions occur that result in seepage of sulfuric acid, sulfate and toxic metals into surface and ground water. The general concept is as follows:

Metal sulfide + air + water →

**Mobilized metal + salts + acid
(including sulfuric acid)**

An example of the metal sulfide reaction is outlined in Figure 1. It demonstrates how sulfuric acid is generated in the presence of unearthed sulfide mineral rock.

Copper-nickel ore frequently contains iron sulfide minerals such as pyrite (FeS_2), one of the world's most common sulfide

FIGURE 2

Acid mine drainage



Iron hydroxide precipitating in a stream can be seen as the yellow-brownish discoloration sometimes referred to as “ochre” or “yellowboy.”

minerals. The atmospheric oxidation of pyrite ultimately results in the release of sulfuric acid.³ Under certain conditions, ferric iron (Fe^{3+}) remains soluble in acidic outflows and forms the reddish-orange to yellow ferric hydroxide ($\text{Fe}(\text{OH})_3$), a precipitate often recognized as the hallmark of waters containing acid mine drainage³ (Figure 2).

Aqueous sulfuric acid is released into the surrounding environment and leaches heavy metals from the rock. The release of sulfuric acid and heavy metals into surface and ground water, and eventually into streams and lakes, is called “acid mine drainage.” Many of the copper sulfide mines currently operating in the United States are located in the Southwest, a region that receives little rain and snow; thus, communication between surface and groundwater resources is limited. In wetter climates like Minnesota's, surface and shallow groundwater are more vulnerable to the negative effects of sulfide mining.⁵

Ore that contains commercially desired metals often contains other metals includ-

ing mercury, lead and arsenic, which are similarly bound to sulfur. Studies of the Duluth Complex formation suggest that leachate will likely include copper, nickel, cobalt and zinc.⁶

By understanding the general concept of sulfide mineral oxidation (Figure 1), one can see how toxic metals are mobilized from solid rock into the environment and can generate sulfuric acid.⁷ This reaction can result in ongoing leaching of metals from mine ore and waste rock, which can continue for centuries.

The Role of Microorganisms

Microorganisms are critical to acid mine drainage, as they accelerate the release of metals. These include extremophilic, sulfur-oxidizing and iron-oxidizing bacteria and archaea. One model organism is *Acidithiobacillus ferrooxidans*, which has been well-studied in the context of sulfide mining because it catalyzes ferrous iron to ferric iron. The regeneration of ferric iron exponentially increases the rate of breakdown of pyrite and sulfide minerals, increasing acid mine drainage.³

Select anaerobic microorganisms carry a gene that allows them to add a methyl group to inorganic mercury to create the most toxic form of mercury, methyl mercury. The environmental conditions that promote mercury methylation are complex and not completely understood, but they often are associated with bacterial sulfate reduction (anaerobic organisms that “breathe” sulfate as an alternative to oxygen).⁸ Methylation occurs in the sediments, wetlands, ombrotrophic (“cloud fed”) bogs and peat lands that are found in Minnesota's water-rich environment.⁹

Multiple variables affect the methylation reaction that creates methyl mercury including pH, temperature and concentrations of carbon, iron and sulfate. It appears that higher levels of sulfate (SO_4^{2-}) can enhance the rates of mercury methyla-

PHOTO COURTESY U.S. GEOLOGICAL SURVEY

tion because they can stimulate bacterial sulfate reduction.⁸ Since acid mine drainage includes sulfate, it is important to understand that increases in sulfate can increase the amount of methylated mercury released into the environment, primarily when that sulfate stimulates bacterial sulfate reduction in anoxic environments.

Mercury Already an Issue in Minnesota

Mercury can be found in the air, sediment, water, soil and living organisms. Humans acquire mercury in two ways: by breathing gaseous mercury or ingesting methyl mercury, notably by eating fish and shellfish. Methyl mercury is found throughout fish tissue, including muscle, and is not removed by trimming the fat, avoiding certain parts of fish or using special cooking methods.¹⁰ Figure 3 shows the sequence of events by which release of anthropogenic sulfate can result in increased mercury levels in fish.

Mercury contamination of fish is a significant public health concern in Minnesota because of its neurotoxicity. In 2011, the Minnesota Department of Health found that one out of 10 infants in Minnesota's Lake Superior region were born with unsafe levels of mercury in their blood.¹¹ Many of Minnesota's northern waters are already legally classified as impaired because of the presence of mercury in fish tissue. This predates any potential mercury increases resulting from acid mine drainage.

Precise predictions of methyl mercury increases that would result from an influx of sulfate caused by mining can be challenging. However, concern is warranted because fishing remains important to Minnesotans,¹⁰ and fish is an important food source for both indigenous and non-indigenous residents. Rural and tribal residents may be at greater risk of mercury exposure than urban or suburban residents because of their higher rates of self-caught fish consumption.¹²

Mercury toxicity as a result of ingesting heavily contaminated fish can result in a range of neuropsychiatric issues including abnormal brain development and sensory distortions (paranoia and hallucinations). The developing brains of fetuses and children can experience the most profound and devastating consequences of exposure to mercury and other heavy metals.

Many illnesses of the brain and central nervous system are categorized as neurodevelopmental disorders. These include attention deficit hyperactivity disorder, learning disorders, autistic spectrum disorders, language disorders and intellectual disabilities. The causes of neurodevelopmental disorders are multifactorial, but the connection to exposures to heavy metals, particularly methyl mercury, is known.¹³

FIGURE 3

Connection between anthropogenic sulfate release and human mercury exposure

Release of sulfate into the environment from anthropogenic source (example: acid mine drainage)

Sulfate reaches wetlands where it can stimulate certain "sulfate-breathing" microorganisms that are capable of converting inorganic mercury to methyl mercury.

Enhanced rates of mercury methylation can occur.

Methylmercury bioaccumulates in aquatic ecosystems, including fish.

Humans consume mercury-contaminated fish.

These conditions cannot be cured, and they come with significant personal, familial and societal costs. A small increase in incidence resulting from increased mercury exposure may result in large costs to society on a population level.¹⁴ A recent consensus statement by Project TENDR issued a strong call for "recommendations to monitor, assess and reduce exposures to neurotoxic chemicals."¹⁵

Air Quality Considerations

The ore complex that contains copper, nickel and precious metals may also contain amphibole fibers. Amphibole fibers are often described as elongated mineral particles (EMPs). EMP fibers are crystals with similarities to asbestos. When ore is mined and processed, EMPs can be released. Currently, EMPs pose an uncertain risk to human health. Because of this uncertainty, longitudinal biomonitoring of people and communities exposed to EMPs is needed.

"Fugitive dust" is a term applied to dust that escapes mining operations. This can include dust that mining trucks generate on the road or dust that escapes as ore is transported in open train cars. Although levels may be difficult to quantify, fugitive dust may have health effects on both mineworkers and residents of nearby communities. Fossil fuel combustion, which is needed to generate electric power for mining, is another source of air pollution, the effects of which need to be considered.

Worker Exposures and Concerns

Safe workplace guidelines are important for people employed in the mining industry. Mine workers require protection from the airborne particulates and dust that are associated with mining operations. Sulfide mining, by virtue of its novel ore composition, presents new environmental safety questions. ➡

The Mine Safety and Health Administration (MSHA) oversees mine safety and releases guidelines for worker protection. MSHA-allowable exposure levels for airborne exposures other than to asbestos are tied to the 1973 American Conference of Governmental Industrial Hygienists (ACGIH) guidelines. The MSHA guidelines do not reflect current science on the health consequences of airborne exposures in mining. The National Institute of Occupational Health and Safety (NIOSH), MSHA and the Occupational Safety and Health Administration (OSHA) have all proposed reduction of the allowable exposure by 50% from the 1973 ACGIH guidelines. In order to better protect Minnesota's miners, the threshold for allowable airborne exposures should be based on more contemporary science. Both NIOSH and ACGIH have published more up-to-date recommendations.^{16,17}

OSHA has published models for medical surveillance of workers exposed to a variety of chemical hazards. Because the Duluth Complex rock includes silicates and other minerals, characterization of the potential adverse chemical and mineral exposures for workers using Duluth Complex-derived rock is important. OSHA provides medical surveillance models for nearly 20 compounds; however, no single overarching medical surveillance recommendation exists for sulfide mining. Given the long latency for the appearance of mining-related health effects, establishment of medical surveillance programs should be considered in the planning of the mine project.

Planning for Unanticipated Events

Proposals for sulfide mining operations must describe how water quality will be preserved, but may not take into account

the extent of extreme weather events. In Minnesota, we are experiencing more significant rain events.¹⁸ In June 2012, for example, the northeastern part of the state received 10 inches of rain in 24 hours. Significant rainfall such as this may result in unintended escape of mining wastewater and accompanying toxins.

A 2015 study of tailings storage facility failures centering on those categorized as "serious" or "very serious" determined that such failures have increased over the last 20 years.¹⁹ For example, in 2014, a British Columbia copper and gold mine tailings pond breach spilled over 6 billion gallons of waste and polluted water into the surrounding lakes and watershed. Such events underscore the need to plan for a catastrophic event involving sulfide mines.

Current regulations also require mining companies to provide plans for the closure of an operation; this involves continued water treatment using filters or reverse osmosis systems. Post-closure water treatment can be necessary for centuries. Equipment malfunctions, natural disasters, extended power outages or inadequate funding can create an unintended interruption in water treatment. It is essential to pre-plan in order to prevent such interruptions from contaminating ground and surface water and the human water supply.

Current Regulation and What is Needed

In 1969, the federal government enacted the National Environmental Policy Act, which directs all federal agencies to take into account the health impacts of all federal actions "significantly affecting the quality of the human environment."²⁰ The Minnesota Environmental Policy Act of 1973 directs "all department and agencies of the state government to ... undertake, contract for or fund such research as is needed in order to determine and clarify effects by known or suspected pollutants which may be detrimental to human health."²¹

With these laws in mind, physicians might assume existing regulations will protect human health. The current mandated evaluations of mining proposals do address air and water quality impacts and toxin discharges. Yet the laws do not require a comprehensive, long-range examination of potential effects on health. For example, environmental reviews may scientifically model the amount of mercury that may be released into surface and ground water, but they do not answer questions about the potential effects on human health of that mercury as it accumulates in food sources.

The short- and long-term effects on human health should be considered in present and future sulfide mining proposals. Both the EPA's Health Risk Assessment (HRA) and Health Impact Assessment (HIA) can be used for this kind of evaluation. The HRA estimates the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environments now and in the future.²² The HIA focuses on "health consequences of decisions upstream from health"²³ and can be defined as "a systematic process that uses an array of data sources and analytic methods and considers input from stakeholders to determine the potential effects of a proposed policy, plan, program or project on the health of a population and the distribution of those effects within the population." The HIA provides recommendations on monitoring and managing those effects.²⁴ Incorporating an HRA and an HIA into the environmental review for a proposed sulfide mining project could enable a more informed, integrated and meaningful discussion of human health concerns.

Conclusion

Sulfide ore mining presents a significant departure from the traditional iron ore mining done in Minnesota. Because of our state's water-rich environment and

the chemical composition of sulfide ore, proposed sulfide mining raises concern about potential deleterious effects on human health. Physicians must continue to educate themselves about the evolving interplay of mining operations and the health of the communities in which they practice. **MM**

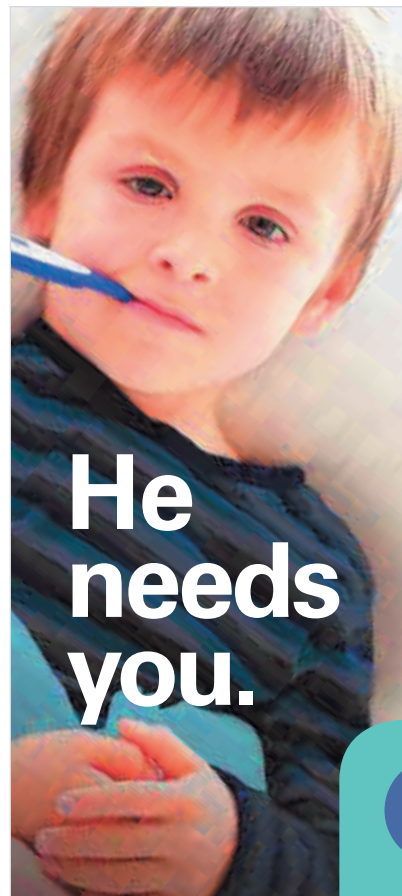
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The authors would like to thank Amy Seip and Caitlin Bakker for their valuable assistance in the preparation of this manuscript.

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Mercury

24 October 2024



Key facts

- Mercury is a naturally occurring element that is found in air, water and soil.
- Exposure to mercury – even small amounts – may cause serious health problems, and is a threat to the development of the child in utero and early in life.
- Mercury may have toxic effects on the nervous, digestive and immune systems, and on lungs, kidneys, skin and eyes.
- Mercury is considered by WHO as one of the top ten chemicals of major public health concern.
- People are mainly exposed to methylmercury, an organic compound, when they eat fish and shellfish that contain the compound.
- Methylmercury is very different to ethylmercury. Ethylmercury is contained in thiomersal as a preservative in some vaccines and does not pose a health risk.

Overview

Mercury occurs naturally in the earth's crust. It is released into the environment from volcanic activity, weathering of rocks and as a result of human activity. Human activity is the main cause of mercury releases, particularly coal-fired power stations, residential coal burning for heating and cooking, industrial processes, waste incinerators and as a result of mining for mercury, gold and other metals.

Once in the environment, mercury can be transformed by bacteria into methylmercury. Methylmercury then bioaccumulates (bioaccumulation occurs when an organism contains higher concentrations of the substance than do the surroundings) in fish and shellfish. For example, large predatory fish are more likely to have high levels of mercury as a result of eating many smaller fish.

Who is at risk?

Mercury is toxic to human health, posing a particular threat to the development of the child in utero and early in life.

All humans are exposed to some level of mercury. Human exposure occurs mainly through inhalation of elemental mercury vapours during industrial processes and through consumption of contaminated fish and shellfish.

It has been estimated that among selected subsistence fishing populations, between 1.5/1000 and 17/1000 children showed cognitive impacts caused by the consumption of fish containing mercury (1).

Signs and symptoms

Mercury is toxic to human health, posing a particular threat to the development of the child in utero and early in life. Mercury exists in various forms: elemental (or metallic); inorganic (e.g. mercuric chloride); and organic (e.g. methyl- and ethylmercury), which all have different toxic effects, including on the nervous, digestive and immune systems, and on lungs, kidneys, skin and eyes.

The health impacts of mercury are numerous including kidney and nervous system damage and skin problems. Exposure of the fetus to methylmercury poses danger to the unborn child.

The inorganic salts of mercury are corrosive to the skin, eyes and gastrointestinal tract, and may induce kidney toxicity if ingested.

Neurological and behavioural disorders may be observed after inhalation, ingestion or dermal exposure of different mercury compounds. Symptoms include tremors, insomnia, memory loss, neuromuscular effects, headaches and cognitive and motor dysfunction. Mild, subclinical

signs of central nervous system toxicity can be seen in workers exposed to an elemental mercury level in the air of 20 µg/m³ or more for several years. Kidney effects have been reported, ranging from increased protein in the urine to kidney failure.

Prevention and control

Interventions to prevent environmental releases and human exposure include:

Eliminate mercury mining, and use of mercury in gold extraction and other industrial processes

Mercury is an element that cannot be destroyed; therefore, mercury already in use can be recycled for other essential uses, with no further need for mercury mining. Mercury use in artisanal and small-scale gold mining is particularly hazardous, and health effects on vulnerable populations are significant. Non-mercury (non-cyanide) gold-extraction techniques need to be promoted and implemented, and where mercury is still used safer work practices need to be employed to prevent exposure.

Promote the use of clean energy sources that do not burn coal

Burning coal for power and heat is a major source of mercury. Coal contains mercury and other hazardous air pollutants that are emitted when it is burned in coal-fired power plants, industrial boilers and household stoves.

Switching to non-mercury thermometers and sphygmomanometers in health care

Mercury has been traditionally used in medical devices, especially thermometers and blood-pressure measurement devices. These represent a hazard in terms of both breakage and long-term disposal. Human exposure occurs mainly through inhalation of elemental mercury vapours. The exposure is entirely preventable with safe and effective mercury-free alternatives.

Implementing safe handling, use and disposal of mercury-containing products and waste

Mercury is contained in many products, including:

- batteries
- measuring devices, such as thermometers and barometers
- electric switches and relays in equipment
- lamps (including some types of light bulbs)
- dental amalgam (for dental fillings)
- skin-lightening products and other cosmetics
- pharmaceuticals.

Dental amalgam, a common filling material for treating dental caries, has been used for over 175 years. One of the global targets of the WHO Global oral health action plan 2023–2030 is that, by 2030, 90% of countries have implemented measures to phase down the use of dental amalgam as stipulated in the Minamata Convention on Mercury or have phased it out.

Skin lightening or skin bleaching is a dangerous practice often associated with the risk of exposure to mercury salts to inhibit melanin production. Mercury-containing skin lightening products are hazardous to health and have been banned in many countries. However, even in countries where tight controls exist, such products may be found being advertised and available to consumers via the Internet and other means.

Mercury use in vaccines and pharmaceuticals

Mercury, such as thiomersal (ethylmercury), is used in very small amounts as a preservative in some vaccines and pharmaceuticals. Compared to methylmercury, ethylmercury is very different. Ethylmercury is broken down by the body quickly and does not accumulate. WHO has closely monitored scientific evidence relating to the use of thiomersal as a vaccine preservative for more than 20 years and has consistently reached the same conclusion: there is no evidence that the amount of thiomersal used in vaccines poses a health risk.

Political agreement

The continued release of mercury into the environment from human activity, the presence of mercury in the food chain, and the demonstrated adverse effects on humans led to the adoption of the Minamata Convention on Mercury in 2013. The Convention obliges Government Parties to take a range of actions, including addressing mercury emissions to air and the phasing out of certain mercury-containing products.

An amendment to the Minamata Convention in 2023 prohibits the manufacture, import or export of certain mercury-added products after 2025. The products list includes batteries, switches and relays, fluorescent lamps, non-electronic measuring devices, cosmetics, etc.

WHO response

The World Health Assembly Resolution 67.11 (2014) on the Public health impacts of exposure to mercury and mercury compounds: the role of WHO and ministries of public health in the implementation of the Minamata Convention requests the Director-General to facilitate WHO's efforts to provide advice and technical support to Member States to support the implementation of the Minamata Convention on Mercury in all health aspects related to mercury.

WHO publishes evidence about the health impacts of the different forms of mercury, guidance on identifying populations at risk from mercury exposure, and tools to reduce mercury exposure.

WHO is committed to raising awareness and understanding of the dangers of mercury exposure to vulnerable populations, such as pregnant women and infants, including online training for health care professionals.

WHO leads projects to promote the sound management and disposal of health-care waste, eliminate use of mercury in skin-lightening products, phase down the use of dental amalgam and is facilitating the development of an affordable, validated, non-mercury-containing medical devices.

WHO is cooperating with the secretariat of the Minamata Convention to develop arrangements for the effectiveness evaluation of the Convention. This is expected to include consideration of human biomonitoring data.

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Mercury Levels in Blood from Newborns in the Lake Superior Basin

GLNPO ID 2007-942

Final Report

November 30, 2011

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Executive Summary

There are considerable data on the levels of mercury in fish from the Lake Superior basin. However, there is limited human biomonitoring, especially in vulnerable populations. Total mercury was measured in dried blood spots from 1465 infants born during 2008 through 2010 to mothers residing in the US portion of the Lake Superior Basin. The purpose of this study was to determine the range of mercury concentrations in these infants and to assess feasibility of using dried blood spots from infants as an indicator of mercury exposure to the fetus and pregnant women. While a regional analysis of mercury exposure data from NHANES reported that the "Midwest" may have lower mercury exposures than other areas of the U.S. (Mahaffey et al., 2009), these data provide evidence of exposures that warrant increased public health action.

A wide range of total mercury concentrations was measured in blood spots. The maximum concentration was 211 µg/l. Of the 1465 samples analyzed 8% were above 5.8 µg/l; the US EPA Reference Dose (RfD) for methylmercury. No association was seen between mercury concentration and sex or urban versus non-urban residence. Results suggest a seasonal exposure pattern with the highest concentrations measured in summer births. While the form of mercury is not known, since total mercury was measured, this seasonal exposure pattern supports a local fish consumption exposure pathway.

Acknowledgments

We thank the Newborn Screening Programs in MN, WI and MI for providing blood spots for this study, Dr. Philippe Grandjean for advice on results interpretation, and Dr. Henry Anderson for consultation throughout the project. Funding for this project was provided by US EPA and the Minnesota Department of Health Environmental Health Tracking and Biomonitoring Program.

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Background

Most mercury exposure occurs through consumption of fish, either sport-fish or commercially available fish (US EPA 1997b). Although there are considerable data on the levels of mercury in fish from the Lake Superior basin there has been limited human biomonitoring for mercury. Currently there are no Minnesota (MN) data on the magnitude or extent of potentially harmful *in-utero* exposures. Measuring total mercury concentrations in newborns' blood within the Lake Superior basin provides information on mercury exposure to the developing fetus. The data collected reinforce the need for health protective outreach and advice on fish consumption.

The Minnesota Department of Health (MDH), in collaboration with state health departments in Wisconsin (WI) and Michigan (MI), measured levels of mercury in the blood of infants born to mothers living within these states respective land areas that drain water into Lake Superior (the "Superior basin"). This project utilized residual dried blood spots (RDBS) from anonymized newborns. Use of newborn RDBS provided a convenient specimen that did not require further sample collection from individuals. The Newborn Screening (NBS) Programs in MI, MN, and WI collected a sample from newborn residual blood specimens (by punching disks from the dried blood spot on the submitted filter paper). This study was reviewed and determined to be exempt by the Institutional Review Boards (IRB) at MDH and the Michigan Department of Community Health. The study consisted of 1465 subjects from the U.S. portion of the Lake Superior Basin. The blood spots were analyzed for total mercury by the MDH Public Health Laboratory (PHL).

Methods

Sampling Design

RDBS from babies born to mothers on the U.S. side of the Lake Superior Basin (residents of MI, MN and WI) were tested for total mercury. The ZIP code of the mother's residence was used to identify eligible blood spot specimens. The geographic boundary of the Lake Superior Basin was defined by watershed boundary data obtained from Natural Resources Research Institute (NRRI 1999). ZIP codes within this boundary were identified using ESRI data (ESRI 2005). ZIP codes with less than one percent land mass within the basin were not included in this study. Figure 1 shows the Lake Superior Basin and ZIP code boundaries within MI, MN and WI.

An anonymized design was used in this study to ensure individuals could not be identified. Data retained for each individual from the NBS data are: state of residence, month and year of birth, sex, and for MN only urban versus non-urban residence as determined by mother's ZIP Code and US Census data.

In MN ZIP codes were categorized as urban or non-urban. Due to the lower number of specimens collected it was not possible to meet the criteria for anonymity (five males and five females per variable) and retain a breakdown of urban/non-urban ZIP codes in WI and MI. Percent urban and percent rural data for each MN ZIP code was obtained from the U.S. Census. At least ten rural-urban classification systems are available for rural health assessment. The US Bureau of the Census maintains definitions of Urban, Urbanized, and Rural Areas for classifying populations. Urban populations are those residing in incorporated areas or Census Designated Places with 2,500 or more or in an Urbanized Area. An Urbanized Area (subset of Urban) is a continuously built up area of 50,000 people or more. A built up area is an area with a population density of more than 1,000 persons per square mile. This is calculated at the census block level. Rural populations are all those not classified as Urban or Urbanized. The definition of urban population is overly inclusive because it includes very small towns. In this study ZIP codes were considered non-urban if the census defined it as less than 65% urban. This cut-off was determined following review of maps and using qualitative community information.

There were differences in sample collection procedures used by each state. MN and WI both started collecting potential participants on a particular date and ended when the quota was reached. The WI NBS Program does not receive maternal address data with the newborn blood spots. To enable sample collection WI Vital Records provided reports of births from residences within the identified ZIP codes to the WI NBS program. The reports included mother's name, date of birth, birth hospital, sex and baby's name. MI selected from days that had the most specimens meeting the ZIP code criteria. Enrollment ran from November 2008 through May 2010 in Minnesota, February 2009 through July 2009 in Wisconsin, and May 2009 through Oct 2009 in Michigan (see Figure 2 and Table 1).

Written, informed consent was obtained before the MDH NBS Program released a residual specimen for use in this study (Figure 2). The NBS database includes all babies screened in MN. After NBS was complete, a weekly query of the NBS data was run to provide a database of potential study participants. This recruitment database provided a running list of specimens with mother's residence ZIP code within the geographic region of the Lake Superior Basin. ZIP codes were used to categorize residences as urban or non-urban.

Informed consent was not sought for all babies from the study area. In an effort to not cause unnecessary additional stress, MDH did not contact parents of babies with complications. Babies were excluded from the mercury study if any of the following criteria were true (some babies fit multiple criteria):

- Families have directed that their child's blood spot card be destroyed.

- Risk factors have been checked on the specimen card including: sick baby, deceased sibling, congenital anomalies, and maternal pregnancy complications.
- Newborn screening results are abnormal.
- Babies known to have been admitted to a Neonatal Intensive Care Unit (NICU) or with a birth weight under 2000 grams
- Deceased babies
- Specimens from St. Mary's hospital in Duluth, MN taken less than 24 hours from birth

Prior to seeking informed consent NBS cards were checked to determine if the residual blood was sufficient for mercury analysis and met quality control requirements (see Sample Collection section below and Appendix A).

MDH began recruiting participants November 1, 2008. Informed consent was not sought prenatally or at the birthing hospital due to concern that these actions could negatively impact participation in NBS. Recruitment initially consisted of sending two letters to mothers followed by three attempts to contact mothers by phone. Phone calling was discontinued in May 2009 due to lack of staffing resources and low rate of success in reaching mothers by phone. The first letter was sent three weeks after birth. If no response was received within three weeks of sending the first letter, a second letter was sent. MDH IRB reviewed the consent process and all materials sent to potential participants. Local Public Health (LPH) assisted with informed consent by requesting consent during home visits. The written communications from MDH informed the mothers that the specimens (if meeting eligibility criteria) would be de-identified. The mothers were instructed that, during and after the research study, MDH would not inform them if their babies' specimen was actually included in the study, of the individual findings, or of the anonymized, aggregate findings. Instead, MDH will generate reports and other communications for scientific audiences and disseminate the anonymized results in a non-targeted manner, such as a posting at the MDH website.

Parents of 2,566 newborns were contacted to request written informed consent. Consent was received from 1130 (44%). Of those who consented 9% were recruited through Local Public Health (LPH). One of the consents received and included in the study, through LPH, was for a baby originally excluded due to risk factors.

The number of samples collected per state was originally designed to be based on the percentage of births in the Lake Superior basin by state. Monthly and annual number of births from 2005 and 2006 were reviewed for MI, MN and WI. Due to changes in the storage, custody, and an unanticipated cost per specimen of the Michigan residual

blood spots the number of specimens from Michigan was reduced from the original plan of 810 to 200. There were 140 specimens collected in WI and 1130 in MN.

Sample Collection

Infant blood is spotted onto filter paper cards 24-48 hours after birth for newborn screening purposes. The Newborn Screening (NBS) Programs in each state (MI, MN and WI) collected a sample from newborn residual blood specimens by punching disks from the dried blood spot on the submitted filter paper. Punches from each specimen were collected and stored in 96 well plates. Each well of the plate was labeled with a unique identifier (Study ID Number) for that specimen. Punches from a blank area from each card was collected in a similar manner and stored in separate wells on the plate containing the corresponding specimen. The blank was used as a quality control measure to check for possible contamination of each specimen card. NBS Laboratories were supplied with pre-populated chain-of-custody forms to track the unique identifiers assigned. The well plates were submitted to MDH PHL for mercury analysis.

NBS cards were excluded from the study if the blood was thought to not produce representative results. The following criteria were established by MDH NBS:

- Specimens other than the initial specimen collected from an infant.
- Specimens collected at greater than 9 days from birth.
- Specimen came from an infant identified as transfused on the specimen card.
- Specimens with more adult hemoglobin than fetal hemoglobin.

Initially eight disks were punched from each NBS card for mercury analysis and a large number of NBS cards were rejected due to insufficient blood. The mercury analysis method was modified and the process was changed to punch 4 disks per NBS card.

The Standard Operating Procedure (SOP) for sample collection, handling, storage, and custody is part of the Quality Assurance Project Plan (QAPP) in Appendix A.

Mercury Analysis

Two 3-mm filter paper disks containing dried blood were placed into a 96-well filter plate to which 0.15 ml of reagent water containing 0.05% 2-mercaptoethanol, 0.001% L-cysteine, 0.005% EDTA, 0.01% Triton X-100, and 10 µg l-1 of Iridium (internal standard) was added, followed by addition of 0.15 ml of 2% hydrochloric acid. The covered filter plate was agitated for 30 minutes and stored overnight at room temperature. The filter plate was re-agitated for 20 minutes and the contents filtered into a 96-well plate and placed on ICP-MS auto sampler for analysis against a five- point aqueous standard calibration curve. For details on the mercury analysis see the SOP for analysis of total mercury in dried blood spots as part of the QAPP in Appendix A.

Periodic surveillance reports and final quality reports were completed by the Quality Assurance (QA) officer. These reports along with the QAPP and SOPs are included in the Appendix. The total number of samples analyzed was 1465 of which 1126 were from MN, 139 from WI, and 200 from MI. Data collected are expressed as a concentration of total mercury in blood (ug/L).

Blind reference material, total mercury concentration 31.4 µg/l, was spotted to filter paper cards and provided to each NBS laboratory. These cards were stored with the participant specimens and punches from these cards were periodically included as a specimen for mercury analysis. These reference samples were blind to the analyst and QA Officer. The results from analysis of these blind references are shown in Figure 4. There is no trend over time, the slope of the regression line is not different from zero (p-value = 0.06), indicating storage conditions did not influence results. The average percent recovery was 80%.

The method used in this study to measure mercury in DBS has not been validated or peer reviewed. The method will be presented at the 2012 Winter Conference on Plasma Spectrochemistry. The rate of quality control acceptance is consistent with other metals analyses performed by MDH. Due to small sample size constraints, it was not always possible to reanalyze samples when quality control samples did not achieve the set criteria thus increasing the number of reported results needing qualification, whereas with other metal analyses, adequate sample volumes allow for reanalysis. In addition, because this is a novel method, extraction efficiency and other performance characteristics of the method were not known prior to this study.

Sixty percent of the reported data are qualified due to low recovery of the Laboratory Control Spikes (LCS). The Quality Control Samples (QCS), LCS, and blind reference samples were all made with National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 966, total mercury concentration of 31.4 +/- 1.7. LCS and reference samples were spotted to filter paper cards and were stored and processed the same as a participant sample whereas the QCS was spiked whole blood. Average percent recovery for QCS, LCS, and reference samples were 86%, 82% and 80% respectively. These recoveries are likely impacted by extraction efficiency. Using all analyses the statistically derived 3-sigma range for the QCS are 70%-101% and LCS are 64% - 98%.

Although the calibration curve was linear for every batch there was a high bias in results for the "medium" and "high" calibration verification standard (CVS). Reported mercury results above the concentration of the medium CVS may be biased high by 10%. Some of the bias could be attributed to the mechanical pipet used to prepare the CVS-medium and the CVS-high verification standards along with the highest calibration standard.

Overall the report level verification was within the limits identified in the QAPP and sensitivity around the report level was good with 7% of the report level verification (RLV) samples analyzed being qualified due to a high recovery and 5% due to a low recovery.

Some of the variability in CVS and RLV results could be due to the use of different lots and suppliers of the standards used. In the method used, the CVS's and RLV were made using a 50:50 mixture of a methylmercury standard and a total mercury standard. This was done to enable future adaptation of the method for speciation. No second source is currently available for the methylmercury standard and its concentration could therefore not be verified. CVS and RLV results from the time period after March 1, 2011 were from a new lot of standards.

Aqueous blanks (AB) were used to assure that reagents were not contaminated with mercury or that carryover of higher levels of mercury was not occurring between the highest calibration standard or LCS and patient samples. Of the 78 batches of samples with reportable results, only one batch had an AB preceding a patient sample that exceeded the MDL.

Method Blanks (MB) for each patient sample punched from their blood specimen spotted onto a filter card, a blank area of the card was also punched to use as a method blank. The method blanks were processed in a manner identical to the associated patient samples. Only one method blank was observed with mercury values over the reporting limit (RL). Sixteen method blanks (1% of blanks analyzed) were estimated to be between the MDL and the RL. Of the sixteen method blanks with low levels of total mercury, only seven (less than 1% of samples analyzed) were observed with mercury values in the blank card punches while those of the accompanying patient sample were free of mercury.

Given the relatively high MDL resulting from limited leftover RDBS material this method is most useful for screening for high exposures. Results reported over the RfD would not be impacted by the reduced sensitivity reflected in the low and high RLV results. High bias of the medium and high CVS impact on results over the RfD may be biased high by about 10%. It is difficult to determine the impact of any bias without understanding the extraction efficiency between inorganic and organic mercury and the standard composition. The manufacturer of the total mercury standard only certifies the total mercury concentration but may contain organic mercury.

Improvements to the method would be a better understanding of the extraction efficiency of the inorganic and organic forms of mercury from dried blood spots. The ability to extract the mercury from blood spots was not known prior to presenting the measurement quality objectives (MQOs) for approval. The assumption that the

extraction efficiencies would match that of aqueous samples was proved to be incorrect. A better understanding of the individual forms could lead to an overall improvement in method extraction efficiency and improved standard recoveries; current method is about 80%. The availability of a second standard for methyl mercury or change the standard used to 100% total mercury rather than a 50:50 mix, since speciation is unlikely due to insufficient leftover DBS.

Mercury Results

A wide range of total mercury concentrations was measured in blood spots from newborns in the US Lake Superior Basin. Forty three percent of the specimens were below the method detection limit (MDL) of 0.7 µg/l. Results between the MDL and the report level (RL) are reported by the MDH PHL as estimated. Estimated values were included in the data analysis. Given the high percentage of non-detects, median (50th percentile) values are reported. Eight percent of the specimens analyzed were above 5.8 µg/l; the US EPA Reference Dose (RfD) for methylmercury (Figure 5). Mercury concentrations in about one percent (14 of 1465) of specimens were above 58 µg/l; the Benchmark Dose Limit (BMDL) used by EPA in developing the RfD. The maximum concentration measured was 211 µg/l. Mercury concentrations were higher in the MN specimens. Using Tukey's test for differences by pairs of geometric means, mercury concentrations in MN specimens are statistically significantly different from WI and MI specimens, but WI and MI are not different from one another (Table 2). Non-detects were assigned a value of the MDL/1.414 for calculating means.

Results suggest a seasonal exposure pattern and therefore support a fish consumption exposure pathway. Births in summer months were higher than other seasons, particularly in MN (Figure 6 and Table 2). Fall and Spring are the only seasons not different from one another; all other seasons are different (overall population & MN only). No association was seen between mercury concentration and sex or urban versus non-urban residence. Differences by birth season, state, and birth month were determined using ANOVA on log-transformed mercury concentration. Tukey's test for differences was used to determine differences between particular pairs of means. Differences by gender and residence were assessed using t tests on log-transformed mercury concentration.

Comparison to other published results

Published data on levels of mercury in newborn blood is not available for comparison. Chaudhuri et al (2009) published an analytical method for measuring mercury in blood spots but did not provide a summary or details on results from mercury analysis of blood spots.

The best available comparison is mercury in umbilical cord blood. Cord blood concentrations have been reported in the range of results from this study. Concentrations up to 735 µg/l were reported from “normal deliveries” (Murata et al., 2007; Tsuchiya et al., 1984). Although there is considerable variability in the relationship, a linear correlation between cord blood and maternal blood has been shown in most studies (Stern, 2005; Stern et al., 2003). Fetal blood mercury has been reported to be, on average, 1.7 times higher than maternal blood (Stern et al., 2003). Given this ratio of cord blood mercury to maternal blood mercury a direct comparison of concentrations in RDBS to adult concentrations is not appropriate. The percentage of participants with mercury levels above the RfD in this study is similar to that for women of childbearing age who participated in National Health and Nutrition Examination Survey (NHANES) (Mahaffey et al., 2009).

Sources of Exposure/Form of mercury

The form of mercury was not determined in this study. Both elemental and organic forms of mercury easily cross the placenta (EPA 1997a). For most people, fish consumption is thought to be the major route of human exposure to mercury (EPA 1997b).

Other possible sources of exposure include dental amalgams and ritual/ homeopathic uses of mercury containing products. Cord blood inorganic mercury has been reported to increase with number of maternal amalgams (Ask et al., 2002). Murata et al 2007 examined studies that measured both methylmercury and total mercury. The mean ratio of total mercury to methylmercury ranged from approximately one to 2.5 suggests other forms of mercury in fetal blood.

Conclusions and Recommendations

The population of focus in the initial sampling design was babies born to mothers residing in the US Lake Superior Basin. Informed consent required in MN and changes in custody of the MI RDBS led to modifications in the sampling design. These changes introduced sampling bias and reduced the generalizability of results. That said, a wide range of mercury concentrations were measured in the large number of specimens analyzed. Of the 1465 participants 8% were above the US EPA RfD for methyl mercury and 1 % were above the BMDL. The results show that mercury exposure is a problem for some in this area. Although a direct link to fish consumption cannot be established from this study, the demonstrated seasonality of exposure provides suggestive evidence of that link. These results provide fish advisory programs stronger evidence for the need to talk with women of childbearing age about reducing mercury exposure. Follow-up studies are needed to determine source(s) of exposure. Increased public outreach and

communication is needed to ensure the public has information that promotes eating fish that are low in mercury.

In cooperation with the GLNPO Project Officer these findings will be disseminated in reports, presentations, and communications with the public. Local, state and tribal public health and health care providers will be notified of these results.

Follow-up studies needed:

Exposure pathways need to be investigated. Screening blood spots for total mercury, as in this study, followed by contact with parent(s) for babies with high levels would be one way to investigate potential exposure pathways. Speciation of mercury in mother's and baby's blood could also be considered.

In addition to exposure investigation there is a need to correlate infant blood spot mercury with cord blood and maternal mercury. Since mercury binds to red blood cells hematocrit/hemoglobin could be also be measured to standardize results.

Are RDBS a useful biomarker of fetal exposure to mercury?

Due to limited availability of blood spots leftover after newborn screening, only two 3mm discs were used for mercury analysis in this study. This increased the MDL and limited characterization of low end of the exposure distribution. Mercury analysis of blood spots is not a routine method; there are refinements that could be made to improve the method.

The method is useful for characterizing the high end of the exposure distribution and for screening for follow-up to determine sources exposure. In order to know the form of mercury a method for speciation needs to be developed. However it is unlikely that there will consistently be enough RDBS to allow for speciation. Speciation could be included when additional blood samples can be collected such as in follow-up after initial screening.

From a policy perspective there is uncertainty regarding future restrictions on use of RDBS for public health surveillance, program evaluation, and public health research.

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Tables

Table 1. Number of Participants by State, Gender, Birth Season, and Residence Type

	Overall (n=1465)	%	MN (n=1126)	%
State			N/A	
MN	1126	77%		
WI	139	9%		
MI	200	14%		
Gender				
Male	730	50%	545	48%
Female	733	50%	581	52%
Unknown	2	0%		
Birth season				
Summer	398	27%	216	19%
Fall	276	19%	227	20%
Winter	370	25%	357	32%
Spring	421	29%	326	29%
Urban/rural	N/A			
Urban			576	51%
Rural			550	49%

Table 2. Mercury Concentrations (µg/L) by Covariates

	Overall (n=1465)		MN (n=1126)	
	Hg GM	Hg median	Hg GM	Hg median
Birth season*				
Summer	1.57	1.23	3.05	2.49
Fall	1.24	0.99	1.38	1.08
Winter	0.68	0.50	0.67	0.50
Spring	1.16	0.91	1.25	1.00
p-value (ANOVA on log values)**	<0.0001		<0.0001	
State			N/A	
MN	1.24	0.91		
WI	0.86	0.76		
MI	0.72	0.50		
p-value (ANOVA on log values)***	<0.0001			
Gender				
Male	1.08	0.80	1.20	0.91
Female	1.15	0.86	1.28	0.94
p-value (t test on log values)	0.20		0.34	
Urban/rural	N/A			
Urban			1.26	0.89
Rural			1.23	0.93
p-value (t test on log values)			0.68	

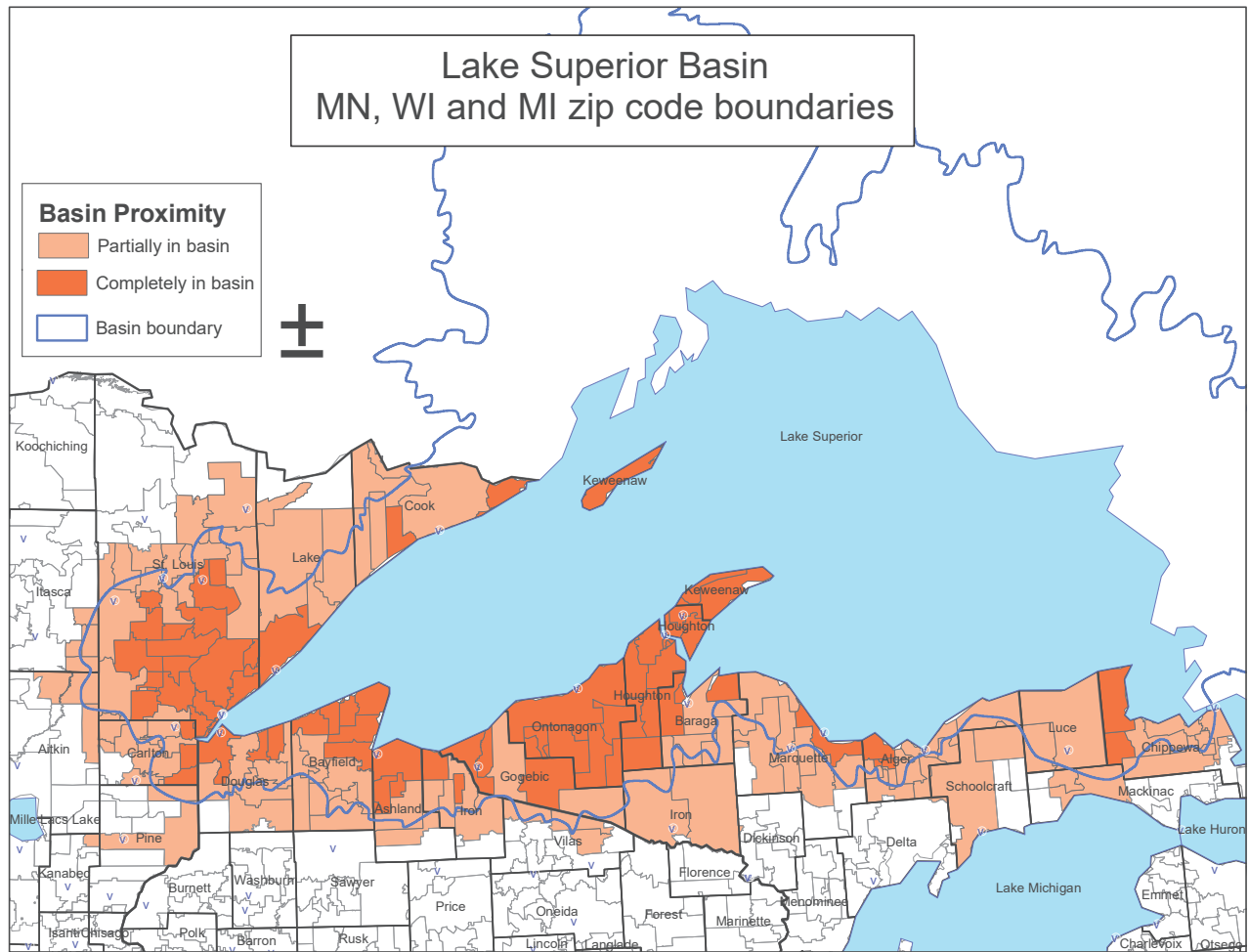
* Based on birth month (Summer = June-Aug.; Fall = Sept.-Nov.; Winter = Dec.-Feb.; Spring = March-May)

**Tukey's test for differences by pairs of means: The only seasons not different from one another are fall and spring; all other seasons are different (overall population & MN only)

***Tukey's test for differences by pairs of means: WI and MI are not different from one another; MN is different from both states

Figures

Figure 1. Lake Superior Basin



Source: ESRI and National Resources Research Institute

Figure 2. Minnesota Informed Consent Process

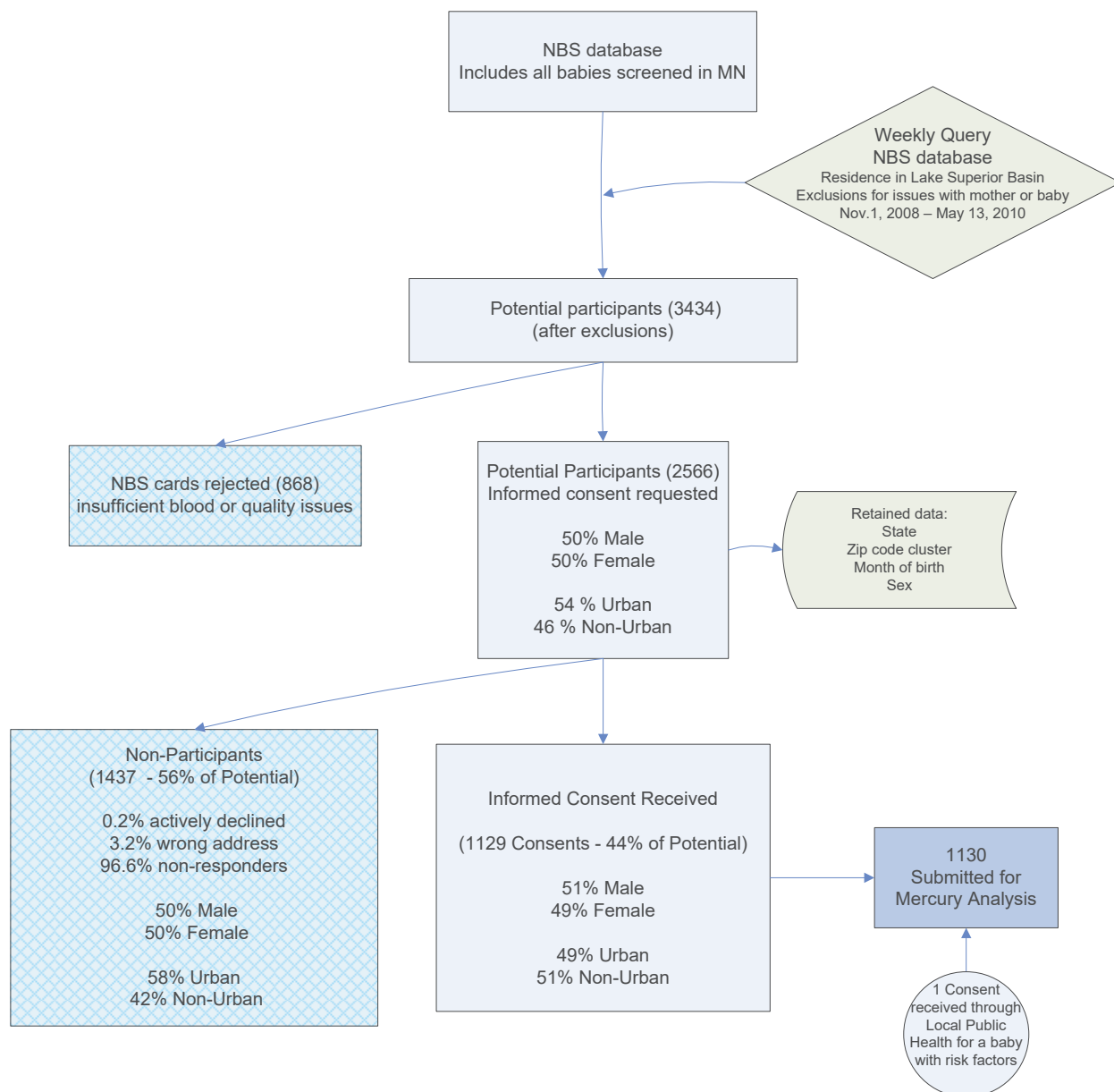


Figure 3. Distribution of Specimens Collected by State, Month and Year

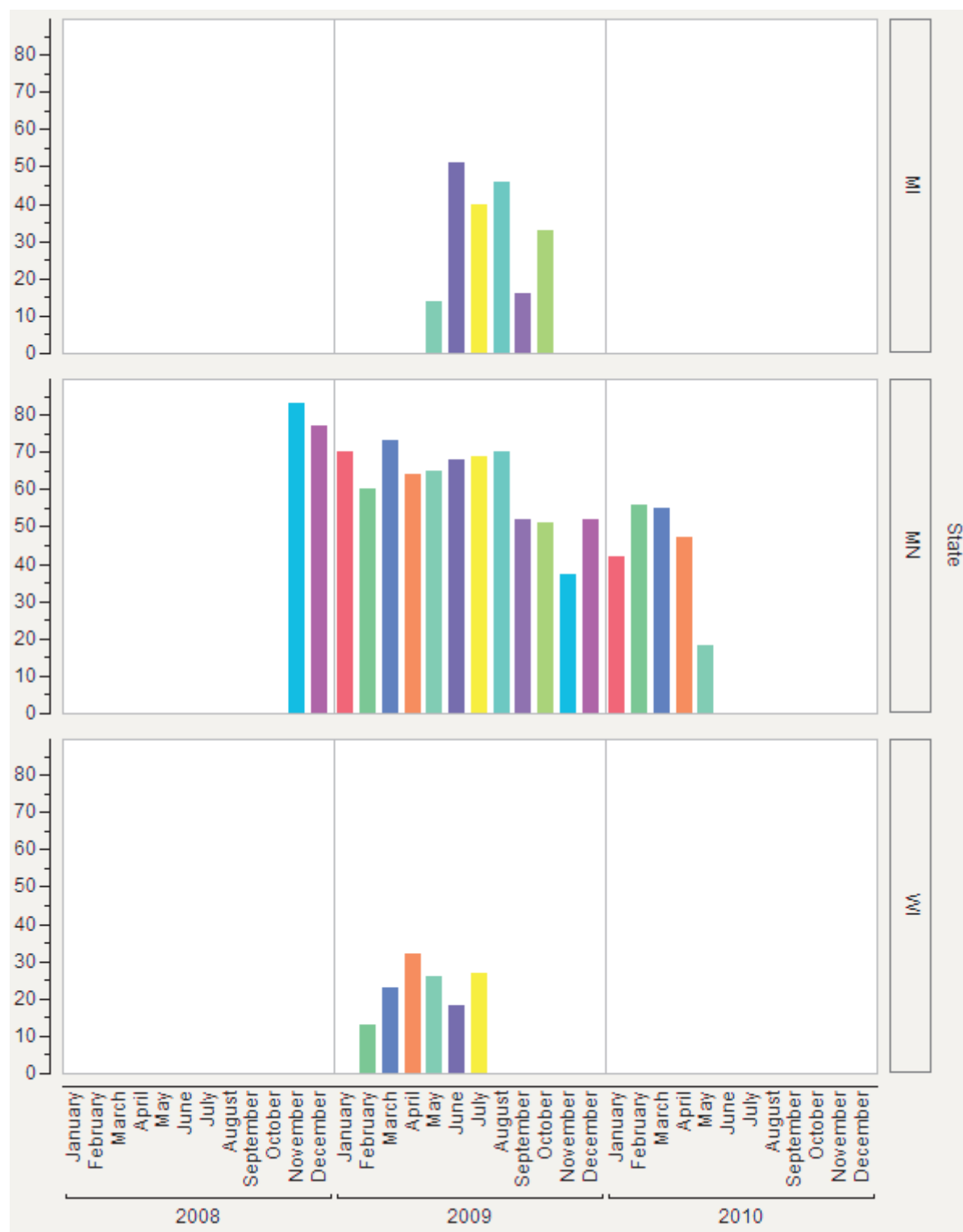


Figure 4. Blind Reference Results

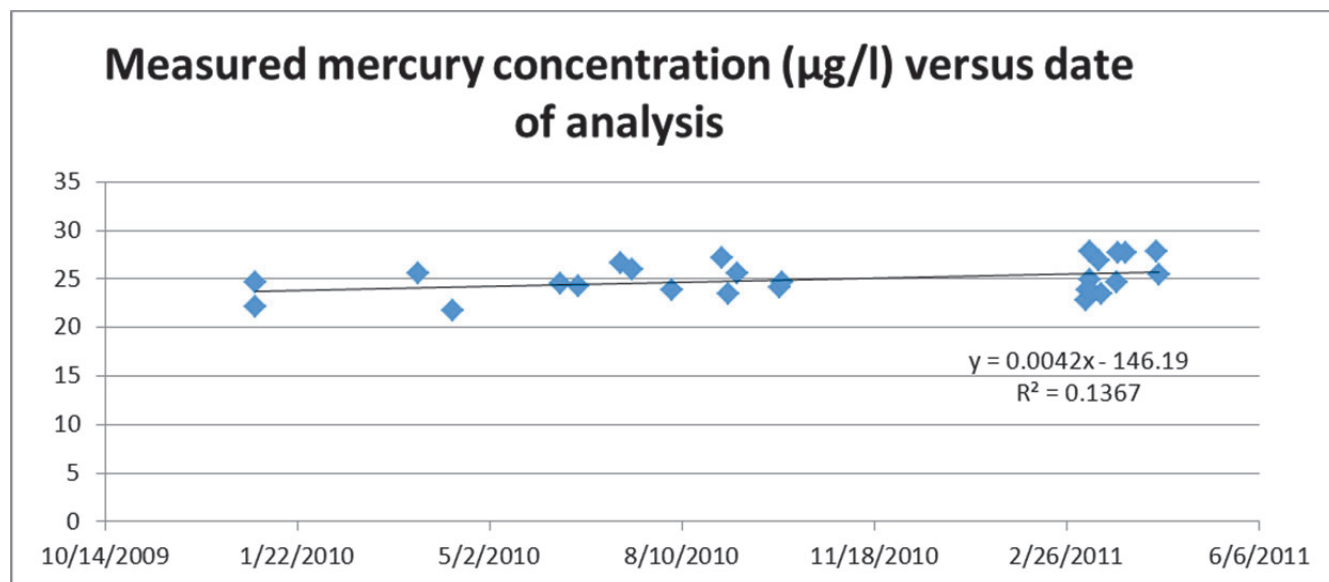


Figure 5. Total mercury in all specimens

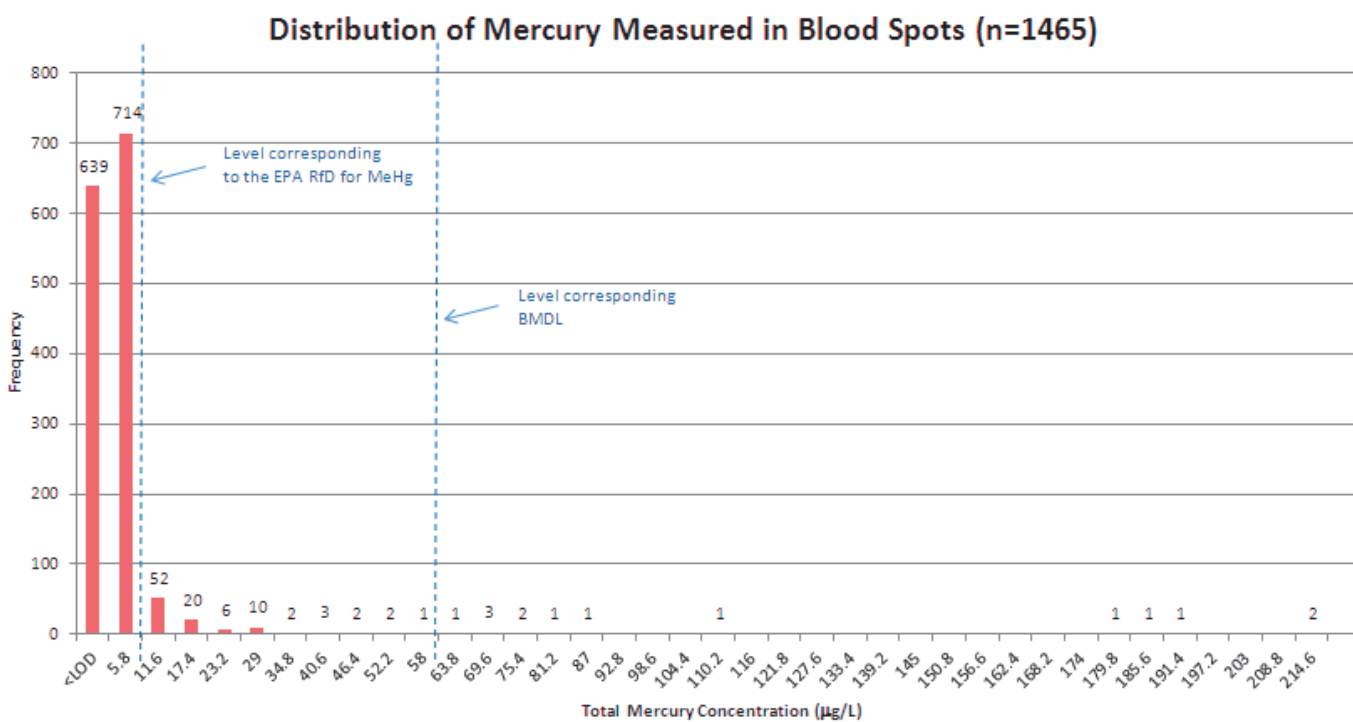
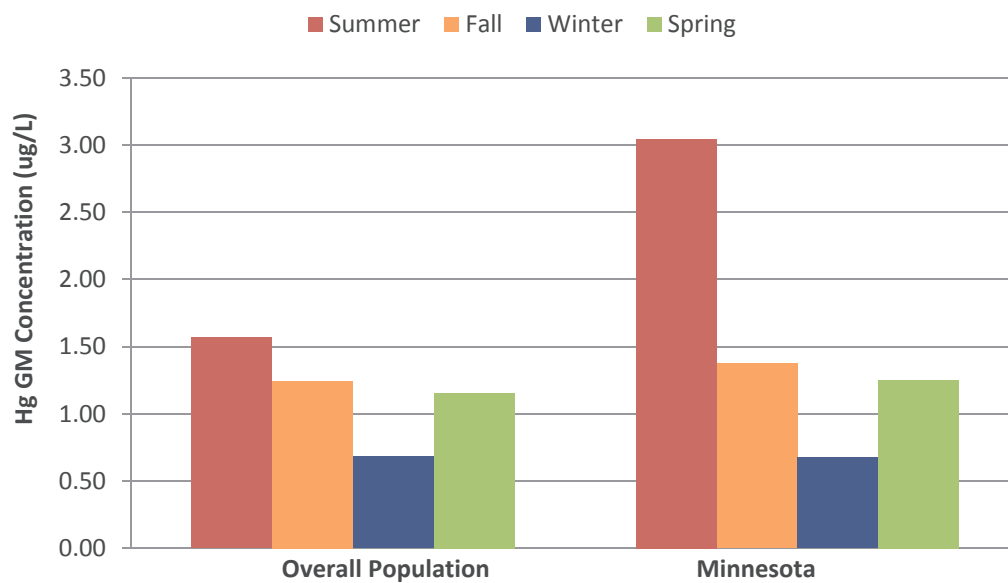


Figure 6. Geometric Mean Mercury Concentration by Season



Appendices

Appendix A: QAPP

Sample Collection SOP

Mercury Analysis SOP

Appendix B: Quality Reports

Surveillance Reports 1-7

Final Quality Report

Final Quality Report Supplemental Information I

Final Quality Report Supplemental Information II

**Mercury Levels in Blood from Newborns
in the Lake Superior Basin
GLNPO ID 2007-942**

Quality Assurance Project Plan

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U.S. EPA Great Lakes National Program Office
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September 2009 – Revision 2

SECTION A. PROJECT MANAGEMENT

A1. Approvals

A handwritten signature in black ink that reads "Patricia McCann". The signature is written in a cursive style with a large, stylized "M".

September 21, 2009

Patricia McCann, Principal Investigator & Project Manager, MDH Date

Jacqueline Fisher, Project Manager, USEPA GLNPO Date

Louis Blume, Quality Assurance Manager, USEPA GLNPO Date

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A3. Distribution List

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A4. Project / Task Organization

The Minnesota Department of Health (MDH), in collaboration with state health departments in Wisconsin (WI) and Michigan (MI), will measure levels of mercury in the blood of infants born to mothers living within these state's respective land areas that drain water into Lake Superior (the "Superior basin").

The Principal Investigator (PI), Patricia McCann, has the responsibility to oversee all aspects of this project. The PI reports to the Health Risk Assessment Supervisor within the Environmental Health Division of the MDH. The PI and MDH Public Health Laboratory (PHL) staff will review all data generated, review the performance of sample handling and sample analyses, and evaluate any necessary corrective action. The PI has the overall responsibility to ensure the quality of all data generated by this project. This Quality Assurance Project Plan (QAPP) addresses the Data Quality Objectives (DQOs) and Measurement Quality Objectives (MQOs) that apply to the data for the project.

The PI will provide interpretation of the data generated by this project in coordination and cooperation with appropriate staff from EPA GLNPO including EPA Great Lakes National Program Office (GLNPO) Project Officer, Jacqueline Fisher, or her designate. The GLNPO Project Officer ensures adherence to the study design, accomplishment of project objectives and approves the project work plan. The QAPP is reviewed and approved by the EPA GLNPO Quality Assurance Officer, Louis Blume.

The MDH Public Health Environmental Laboratory Manager directs a major public health laboratory with multiple and varied functions and statewide impact involving considerable scientific or technical complexity. The duties include planning, implementing, and evaluating the application of fiscal, human, and technical resources to respond to the state's current and long-range projected analytical needs.

The MDH Public Health Environmental Laboratory Inorganic Chemistry Unit Supervisor supervises analytical and research activities in the Inorganic Chemistry Unit of the Environmental Laboratory. The duties include hiring, training, directing, and evaluating unit staff, and planning and directing quality assurance systems within the unit.

The MDH Public Health Environmental Laboratory Research Scientist performs scientific research in the Inorganic Chemistry Unit of the Environmental Laboratory. The duties include planning, conducting, and evaluating the research, and participating in writing reports and publications.

The MDH Public Health Environmental Laboratory Quality Assurance (QA) Officer leads the laboratory staff in implementing, maintaining, and documenting the laboratory's quality system. The duties include training staff in quality assurance, evaluating data quality and technical proficiency, and responding to client needs and complaints.

Organizational charts for MDH, the Public Health Laboratory, the Public Health Environmental Laboratory, and the Environmental Health Division are in Appendix D.

A5. Problem Definition / Background

Everyone who eats fish, either sport-fish or commercially available fish, is exposed to mercury. Although there are considerable data on the levels of mercury in fish from the Superior basin there has been limited human biomonitoring for mercury. Currently there are no Minnesota data on the magnitude or extent of potentially harmful *in-utero* exposures.

This project utilizes residual dried blood spots (DBS) from newborns. Use of newborn DBS provides a convenient readily available specimen that does not require further sampling from individuals.

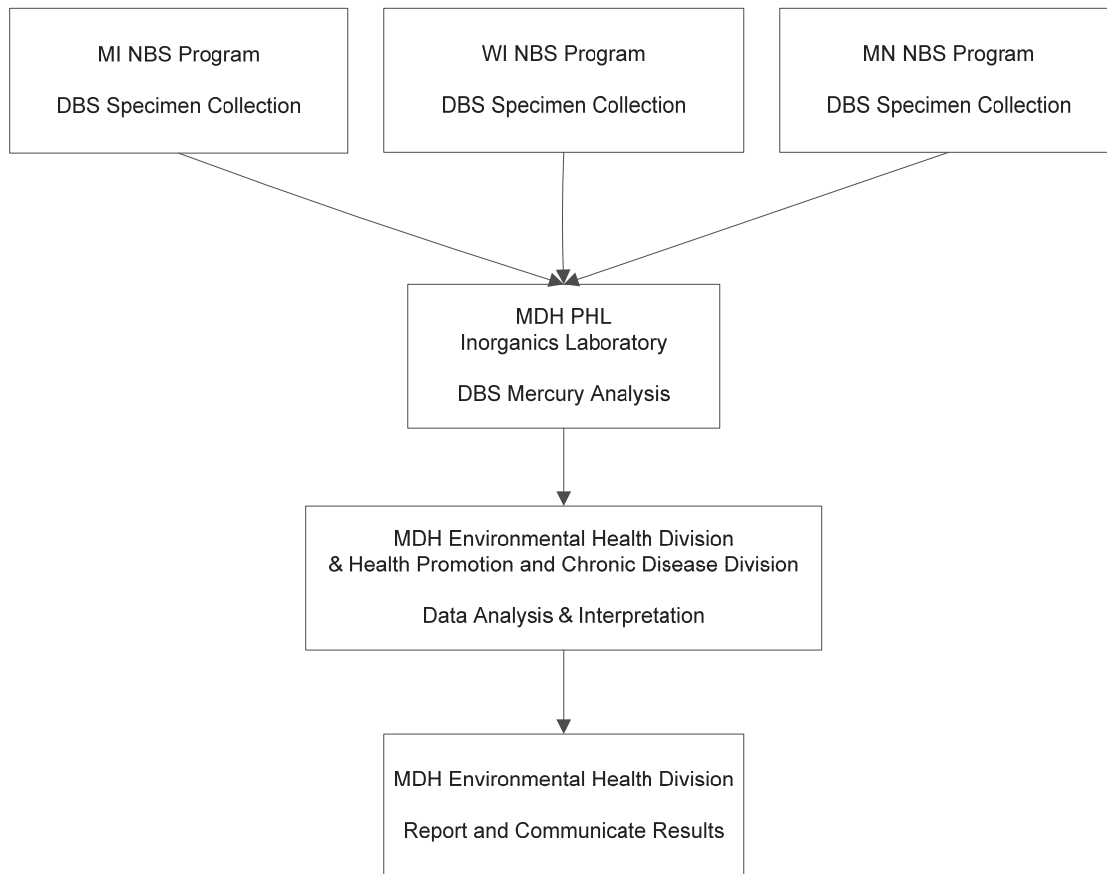
Measuring total mercury concentrations in newborns' blood will help characterize exposure to mercury in Minnesota and assist in directing outreach on fish consumption advice. People are exposed to mercury through consumption of fish. Measuring total mercury concentrations in newborns' blood within the Superior basin will help characterize this population's exposure to mercury. The data collected will assist public health departments in targeting health protective outreach and advice on fish consumption, which is the major source of mercury exposure. Public health agencies will also use these data to provide primary care providers with direction on targeting subpopulations.

A6. Project / Task Description

The Newborn Screening (NBS) Programs in each state (MI, MN and WI) will collect a sample from newborn residual blood specimens (by punching disks from the dried blood spot on the submitted filter paper). The study will consist of approximately 1500 subjects from the U.S. portion of the Lake Superior Basin. The blood spots will be analyzed for total mercury by the MDH PHL. The data collected will be expressed as a concentration of total mercury in blood (ug/L). MDH will analyze the data and report the results.

Sample collection and analysis will begin after the QAPP has been approved. Sample collection is expected to begin in the fall of 2008. Factors that will be considered in determining the work schedule are availability of samples and the availability of instrument and analyst time. The project will be completed and the final report submitted by September 30, 2010.

Figure 1. Project Participants and Flow Diagram



A7. Data Quality Objectives for Measurement Data

Data Quality Objectives (DQOs)

The sensitivity of the method selected to analyze total mercury in dried blood spots should provide a reporting level no greater than 5.8 ug/L mercury in cord blood (CDC 2004), the level that has been identified in the literature as a reference dose. Individual cards containing dried blood spots may vary in contamination, therefore, the data used to assess the project must also measure any potential contamination that may arise from handling samples and cards in varying hospital and laboratory environments, as well as in transport and storage.

The MDH standard operating procedures (SOPs) for sampling and analysis found in Appendix B and C will potentially produce data that meet the requirements of the project.

A summary of the performance criteria of the MDH analytical SOP is listed in Table 1.

Table 1. Measurement Quality Objectives (MQOs)

Requirement	Sample Code	Acceptance Criteria (MQO)
Reporting Units	ug/L total mercury in blood	
Instrument detection limit Frequency Criteria	IDL (Instrument Detection Limit)	Once per project
Method detection limit Frequency Criteria	MDL (Method Detection Limit)	Once per project 0.79 ug/L total mercury in blood (calculated from DBS)
Reporting level verification Frequency Criteria	RLV (Reporting Level Verification)	Spiked human blood as DBS on card Once per analytical run 2.4 ug/L total mercury in blood (calculated from DBS) Results between the MDL and RLV will be flagged as estimated values.
Calibration Verification Frequency Criteria	CVS (Calibration Verification Standard)	Aqueous standard at high, medium, low concentrations Beginning of run, every 20 samples, end of run Within 2S, or reject samples analyzed back to the last passing CVS if one average mean is outside 3S, two average means are outside 2S on same side of mean, average of high, medium, and low means are outside 2S on opposite side of mean, or current and previous 9 run means are on same side of the characterization mean for either the high, medium, or low
Quality Control Sample Frequency Criteria	QCS (Quality Control Sample)	NIST SRM 966 Beginning of run, every 20 samples, end of run 80-120%
Laboratory Control Sample Frequency Criteria	LCS (Laboratory Control Sample)	NIST SRM 966 on card Beginning of run, every 20 samples, end of run 80-120%
Reference samples Frequency Criteria	RS (Reference Sample)	NIST SRM 966 on card sent blind to MDH laboratory Periodically throughout the study 80-120%
Internal standard Frequency Criteria	Rh (Rhodium, the internal standard)	Every sample, blank, standard +/- 50% of the average Rh value of the calibration standard of that run

Requirement	Sample Code	Acceptance Criteria (MQO)
Blanks Aqueous Blank Frequency Criteria	AB (Aqueous Blank)	All aqueous reagents Beginning of run immediately after calibration, after every 20 samples, end of run < MDL
Method Blank Frequency Criteria	MB (Method Blank)	Spots punched from blank part of each specimen card Every sample < MDL, blank and associated data flagged if >MDL
Duplicates Field Duplicates Frequency Criteria	FD (Field Duplicate)	Spots punched in duplicate from patient sample One per 20 samples +/- 20% RPD when concentration >10x MDL, when concentration <10x MDL then +/- RL
Laboratory Duplicates Frequency Criteria	LD (Laboratory duplicate)	Replicate analysis of patient sample Beginning of run, End of run 80-120%

A8. Special Training Requirements/Certification

Laboratory personnel are trained to perform the functions required of the project.

Laboratory personnel from the three state newborn screening laboratories are experienced and familiar with the procedures of selecting and excluding sample specimens. The personnel have been trained in and will comply with the specific requirements in the SOPs for the project.

Laboratory personnel from the MDH environmental laboratory are trained in the policies and procedures of the laboratory relating to analysis, quality assurance, and data management as described in the MDH Quality Assurance Manual (QAM), Section 5.0 Personnel. The MDH QAM is found in Appendix C.

The Research Scientist responsible for the mercury analysis has been trained in the calibration and use of the ICP-MS instrumentation. The analyst participates in proficiency testing programs coordinated through the Centers for Disease Control and Prevention (CDC) that provide challenge samples for mercury in human blood. The MDH Public Health Laboratory has CLIA certification; specifically, the analysis of human blood for mercury is included in the CLIA inspections for compliance with toxicology procedures.

A9. Documentation and Record

The MDH Environmental Laboratory will maintain documentation of project activities in records including laboratory notebooks, instrument (raw) data files, and final processed data (in spreadsheets or printouts from the instrument). Quality control information will be recorded in notebooks and printouts in the same format used for sample results. An electronic inventory of all samples received and their progress in the laboratory is also

maintained. These files are available for review on site by the EPA Project Officer or QA Officer.

It is the analyst's responsibility to check the QC information against the limits for the analysis. When the Research Scientist determines that a batch of samples is not in control because the acceptance criteria have not been met, the analyst will immediately bring the matter to the attention of the laboratory's Inorganic Chemistry Unit Supervisor. The supervisor and/or analyst will document out-of-control analyses and will file a corrective action report with the laboratory QA Officer.

The turn-around-time for sample analyses will be agreed on prior to collecting the first samples for the project. Factors that will be considered in determining the turn-around-time are availability of samples and the availability of instrument and analyst time.

The MDH Environmental Laboratory will provide final data reports to the Principal Investigator. At a minimum, the laboratory will provide the following:

- Original chain of custody
- Analyte report level
- Quality control limits
- Client sample identifier
- Laboratory sample identifier
- Date the sample was punched from the specimen card
- Date the sample was received at the laboratory
- Date the sample was analyzed
- Method identifier
- Concentration of total mercury in the sample
- Units used for expressing the analyte concentration
- Qualifications or comments that provide additional information on the analysis
- Table of results of method blanks analyzed over the course of the study
- Table of results of laboratory duplicate sample analyses analyzed over the course of the study
- Table of percent recoveries for laboratory control samples analyzed over the course of the study
- Table of reference of samples and QC determinations in each analytical batch

Error Handling. If an error is made during observations made in the laboratory, corrections will be made by crossing through the error with a single line so that the original entry is visible. The correct information will be entered next to the crossed out error. All corrections will be initialed and dated. Errors in reporting are documented through amended reports and/or corrective action reports submitted and approved by the laboratory QA Officer and/or Environmental Laboratory Manager.

Reporting Analytical Results. Analytical results will be reported to the Principal Investigator by the laboratory as the results are generated. A discussion on anomalies will be included in the laboratory's final report. At the conclusion of the study, a tabulated

summary of all the sample batches analyzed will be provided in a spreadsheet format to the Principal Investigator. The tabulated data will identify the sample number, the analytical result, the batch identification, and the results of all quality control determinations analyzed in the batch. Copies of the final reports and the tabulated data summaries will be retained by the laboratory.

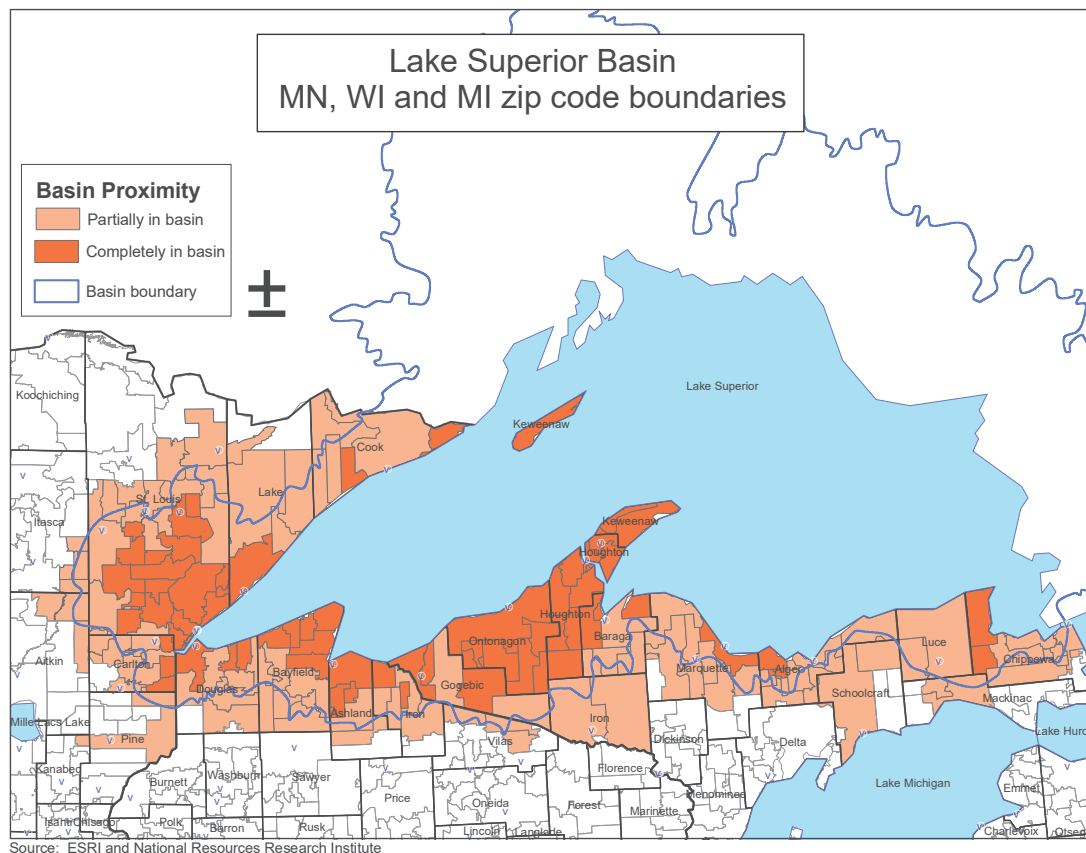
Data Storage and Retention. All project records, including chain of custody forms, raw analytical data, project-specific corrective action reports, project correspondence, and project reports, will be maintained by the laboratory for a minimum of 1 year at the laboratory and for the additional years at an off-site record storage location maintained by the State of Minnesota for a total of 10 years. The laboratory will maintain computer hardware and software to allow computer-acquired/generated data and text files to be read for 10 years after collection/generation. When records are transferred to the off-site location, an inventory of the records will be maintained.

SECTION B. DATA GENERATION AND ACQUISITION

B1. Sampling Process Design

Residual DBS from babies born to mothers on the U.S. side of the Lake Superior Basin (residents of MI, MN and WI) will be tested for total mercury. Enrollment began on November 1, 2008 in Minnesota, February 1, 2009 in Wisconsin and will likely begin in the fall of 2009 in Michigan. The ZIP code of the mother's residence will be used to identify blood spot specimens. The geographic boundary of the Lake Superior Basin was defined by watershed boundary data obtained from Natural Resources Research Institute (NRRI 1999). ZIP codes within this boundary were identified using ESRI data (ESRI 2005). ZIP codes with less than one percent land mass within the basin will not be included in this study. Figure 2 shows the Lake Superior Basin and ZIP code boundaries within MI, MN and WI.

Figure 2. Lake Superior Basin



The WI NBS Program does not receive maternal address data with the newborn blood spots. WI Vital Records will provide reports of births from residences within the identified ZIP codes to the WI NBS program. The reports will include mother's name, date of birth, birth hospital, sex and baby's name.

ZIP codes will be categorized as urban or non-urban. Percent urban and percent rural data for each ZIP code was obtained from the U.S. Census. At least ten rural-urban classification systems are available for rural health assessment. The US Bureau of the Census maintains definitions of Urban, Urbanized, and Rural Areas for classifying populations. Urban populations are those residing in incorporated areas or Census Designated Places with 2,500 or more or an Urbanized Area. An Urbanized Area (subset of Urban) is a continuously built up area of 50,000 people or more. A built up area is an area with a population density of more than 1,000 persons per square mile. This is calculated at the census block level. Rural populations are all those not classified as Urban or Urbanized. The definition of urban population is overly inclusive because it includes very small towns. In this study will be considered non-urban if the census defined it as less than 65% urban. This cut-off was determined following review of maps and using qualitative community information.

ZIP codes categorized as non-urban will be lumped together by state. Lumping is necessary for anonymity given the low birth rate by ZIP code within the basin, particularly for non-urban ZIP codes.

The number of samples per state was originally based on the number of births in the basin by state. Monthly and annual number of births from 2005 and 2006 were reviewed for MI, MN and WI. Due to changes in the storage, custody, and cost per specimen of the Michigan residual blood spots the number of specimens from Michigan will be reduced from the original plan of 810 to 200. The total number of specimens collected by all three states will be approximately 1500.

B2. Sampling Methods

The Newborn Screening (NBS) Programs in each state (MI, MN and WI) will collect a sample from newborn residual blood specimens (by punching disks from the dried blood spot on the submitted filter paper).

The Newborn Screening Laboratories will use the following criteria to select blood specimens for inclusion in the study:

- Mother's residence reported on birth certificate is within the Lake Superior Basin
- The specimen must not be a repeated specimen.
- A sufficient amount of blood must remain for newborn screening activities.
- The selected specimens must conform to requirements of the Clinical and Laboratory Standards Institute's Simple Spot Check method.

- MN specimens: No risk factors checked on the specimen card which include: sick baby, deceased sibling, congenital anomalies, and maternal pregnancy complications, and all of the newborn screening results are normal.

Specimen punches from each patient will be collected and stored in 96 well plates. Each well of the plate will be labeled with a unique identifier (Study ID Number) for that specimen. Punches from a blank area from each card will be collected in a similar manner and stored in separate wells on the plate that contains the corresponding patient specimen. The blank will be used as a quality control measure to check for possible contamination of each specimen card. Laboratories will be supplied with pre-populated chain-of-custody forms to track the unique identifiers assigned.

Written, informed consent will be required before the MN Newborn Screening Program can release residual specimens for use in this study. Mothers will be contacted a few weeks after their infant's birth and asked for their written, informed consent. The written communications from MDH, which the Minnesota mothers will receive at the outset of the study, will inform the mothers that the specimens (if meeting eligibility criteria) will be de-identified. The mothers will be instructed that, during and after the research study, MDH will not inform them if their babies' specimen was actually included in the study, of the individual findings, or of the anonymized, aggregate findings. Instead, MDH will generate reports and other communications for scientific audiences and disseminate the anonymized results in a non-targeted manner, such as a posting at the MDH website.

The MDH SOP for sample collection, handling, storage, and custody is found in Appendix A.

B3. Sample Handling and Custody

Samples from Michigan and Wisconsin will be delivered to the Environmental Accessioning area of the MDH Public Health Laboratory through the U.S. postal system. Samples from Minnesota will be hand-delivered to the Environmental Accessioning area of the MDH Public Health Laboratory. Shipments, whether by mail or delivered in person, will be accompanied by the pre-populated chain-of-custody form associated with the samples delivered.

Samples will be held at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ prior to shipment. Specimens will be shipped with ice packs to maintain a temperature $<10^{\circ}\text{C}$ during transit.

The MDH SOP for sample collection, handling, storage, and custody is found in Appendix A.

B4. Analytical Methods Requirements

Filter paper disks containing dried blood are treated with acidic reagents in a clean room facility to release and recover total mercury for analysis. The reagent solution contains an internal standard. After vortexing the disks and allowing them to soak overnight in the reagents, the eluant is analyzed using an ICP-MS.

The laboratory's Research Scientist and Inorganic Chemistry Unit Supervisor are responsible to assure that the requirements of the method are met. If method requirements are not met, or other non-conformances are encountered, the analyst and Inorganic Chemistry Unit Supervisor must stop the analysis, locate the problem, and take corrective actions prior to resuming sample analysis.

The MDH SOP for analysis of total mercury in dried blood spots is found in Appendix B.

B5. Quality Control Requirements

Quality Control (QC) determinations shall be used to measure the attributes and performance of processes both to prevent and identify sources of error. The MQOs listed in A1 Table 1 summarize the types, frequencies, and performance criteria that will be used to assess the quality of the sample analyses of the project.

Sample Collection Quality Control

Prior to collecting samples for the study, the MDH Environmental Laboratory will assure that the three punching devices supplied to the newborn screening laboratories in Michigan, Wisconsin, and Minnesota produce uniform-sized punches.

Filter paper blanks will be used during the project to assess whether blood specimens mercury levels are biased due to contamination of the card on which the blood was collected.

Duplicate samples will assess the precision of the method and may demonstrate that the blood specimens selected for punching contained uniformly distributed blood.

Studies conducted by the MDH Newborn Screening Laboratory have documented that the volume of blood spotted onto filter paper and punched into a 3 mm circle is 3.1 uL. MDH Environmental Laboratory will use this relationship to calculate the concentration of blood in sample specimens.

Chain-of-custody forms will be pre-populated with unique sample identifiers prior to sending the forms to the sample collection laboratories in order to minimize the possibility of using the same number for two separate specimens.

Analytical Quality Control

Scheduled quality control determinations for the analytical procedures include sample card blanks punched from each specimen card in which blood spots are punched, performance blood samples, internal standards, single-blind punched blood spots randomly distributed among field samples, field duplicates, and laboratory duplicates.

Evaluation procedures will be used to assess the QC data generated. The evaluation procedures include measurements for precision, accuracy, sensitivity, quantitation limits, completeness, representativeness, and comparability. The definitions and equations for data manipulations are described below.

Precision

Precision is a measurement of mutual agreement (or variability) among individual measurements of the same property, usually under prescribed similar conditions. Precision will be assessed using field duplicates and laboratory duplicates. Precision is calculated in terms of relative percent difference (RPD). The RPD of each duplicate is compared to the laboratory-established RPD for the analysis. The Research Scientist or the Inorganic Chemistry Unit Supervisor must investigate the case of data outside stated acceptance limits. Corrective action may include recalibration, reanalysis of QC and field samples (if sample volume is available), or flagging the data as suspect if the analysis cannot be repeated.

The following equation is used to evaluate precision:

$$RPD = \frac{|S - D|}{(S + D)/2} \times 100$$

Where:

S = first sample value

D = second sample value

The acceptance limit for field duplicates with concentrations greater than ten times the method detection limit is $\pm 20\%$ RPD.

The acceptance limit for field duplicates with concentrations less than ten times the method detection limit is $\pm$ report level.

Accuracy

Accuracy is the measure of the bias in a measurement system or the degree of agreement of a measurement with an accepted reference of “true” value. Accuracy will be assessed using reference samples. The reference samples consist of NIST Standard Reference Materials 966 which contains toxic metals, including mercury, in bovine blood. Accuracy is calculated in terms of percent recovery. The percent recovery of each duplicate is compared to the laboratory-established minimum and maximum recoveries expected for individual measurements for an in-control system. The Research Scientist or the

Inorganic Chemistry Unit Supervisor must investigate the case of data outside stated acceptance limits. Corrective action may include maintenance, recalibration, reanalysis of QC and field samples (if sample volume is available), or flagging the data as suspect if the analysis cannot be repeated.

The following equation is used to evaluate accuracy:

$$\% R = X/T \times 100$$

Where:

% R = percent analyte recovery

X = measured value

T = true value

Background Assessment

Two types of blanks will be used to assess contamination and low system background: sample card blanks and laboratory blanks.

Sample card blanks consist of an equivalent number of spots punched from the blank part of each specimen card. They are used to assess contamination from the card, sample containers, and equipment and disinfection techniques used during the protocols followed in punching the dried blood spots. All contaminant concentrations should be less than the reporting level. Sample results will not be corrected for sample card blank values. Analyte concentrations in the samples and blanks will be reported and the blank flagged if it exceeds the reporting level.

Laboratory blanks consist of aliquots of reagents used to make up calibration standards and performance standards. Laboratory blanks are used to assess contamination that may arise from procedures within the laboratory. All contaminant concentrations should be less than the reporting level.

Sensitivity

Sensitivity will be evaluated using the instrument detection limit, the method detection limit, and the reporting level.

The instrument detection limit (IDL) is determined by calculating the average of the standard deviations of three analytical runs performed on non-consecutive days of reagent blank solution with seven consecutive measurements per day. The IDL is determined at the beginning of the project to demonstrate that analyte response is greater than the background noise of the instrument. The IDL is not used for quantitation of method analytes.

The method detection limit (MDL) is determined by the standard deviation of the concentration of mercury found in dried blood spots of human blood spiked with mercury

at the lowest concentration of the calibration curve. The MDL is determined at the beginning of the project to establish the minimum concentration above which sample values will be reported. In data aggregation, processing, and interpretation, values below the MDL are considered as zero.

The reporting level for the project will be the lowest standard in the calibration curve, which is equivalent to 2.4 ug/L total mercury in blood calculated using the volume of blood equivalent to two dried blood spots.

Completeness

Completeness is the amount of valid data obtained from a measurement system compared to the amount that was expected to be obtained under correct normal operations. Completeness is calculated in terms of the valid data percentage of the total tests requested. Valid analyses are defined as those where the sample arrived at the laboratory intact, in sufficient quantity to perform the requested analyses, accompanied by a completed chain of custody form, and analyzed in such a manner that analytical QC acceptance criteria are met. Our QA objective for completeness is 95%.

Representativeness

Representativeness expresses the degree to which data accurately and precisely represents a characteristic of a population or sampling point. The characteristics of representativeness are usually not quantifiable. Sampling technique and sampling size have been carefully chosen to reflect a high degree of representativeness. Samples are expected to be very homogeneous and the spots selected from the sample container are expected to be representative of the entire sample.

Comparability

Comparability expresses the confidence with which one data set can be compared to another data set measuring the same property. Comparability is ensured through the use of established SOPs, consistency in analytical and sampling personnel, consistency in analytical technique, consistency in reporting units, and use of traceable standards for instrument calibration. When data are judged to be representative and when precision and accuracy are known, the data sets can be compared with the highest degree of confidence.

Data from this project will not be compared to data generated by other laboratories; therefore a measure of inter-laboratory comparability can not be estimated.

B6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements

The laboratory will maintain instruments and equipment in sound operation condition and working order. Preventative maintenance and repairs are documented in dedicated log books. The laboratory maintains a supply of spare parts at the instrument, including spare cones, tubing, torch, and spray chamber. Service contracts are in place on the ICP-MS instrument and all major equipment to assure repairs, if needed, are made in a timely manner.

Balances are calibrated annually on-site to NIST-traceable standards by a contracted calibration company. Balances are checked daily prior to first use by weighing one or more known masses.

Mechanical pipettes are calibrated semi-annually by a contracted calibration company to ensure accurate and precise delivery of measured volumes of standards.

The sensitivity, precision of replicate injections, mass calibration, mass resolution, and low system background of the ICP-MS is evaluated each day of use by analyzing and examining the results of a tuning performance standard prior to analyzing samples. Any deviation requires stopping the analysis and performing a complete evaluation of the instrument, including repairs if necessary. If the ICP-MS is shut down for maintenance or repair, it must be tuned prior to analyzing samples using the tuning performance standard. All tuning observations are documented.

At least twenty punched disks from the punching devices used to prepare the dried blood spots are weighed and the weights compared prior to first use by the Newborn Screening laboratories in MI, MN, and WI to assure that the individual punching devices produce relatively uniform sized punches. The performance criterion is based on the statistical evaluation of differences between the means of the weights obtained from the sample sets. Once passed for use in the study, no further inspection or maintenance will be conducted on the punching devices.

B7. Instrument/Equipment Calibration and Frequency

The ICP-MS is calibrated with every use and prior to sample analysis by analyzing a calibration blank and five calibration standards. The calibration blank consists of all reagents used on samples along with two punches of blank filter card. The calibration standards are five aliquots of reagents that have been spiked in the laboratory with varying concentrations of mercury along with two punches of blank filter card. The range of calibration corresponds to total mercury in blood from 2.4 ug/L to 48 ug/L calculated using the volume of blood equivalent to two dried blood spots. A calibration curve is developed using a linear regression of concentration vs. response. All calibration standards are prepared from solutions traceable to certified standards. The calibration curve is verified against a second-source standard. Both the calibration curve and the verification must meet the established acceptance criteria. If the results are outside the acceptance criteria, corrective actions are performed. The acceptance criteria are defined in the MDH SOP for total mercury in dried blood spots. Sample results from unacceptable calibrations or unacceptable calibration verifications are not reported.

Hard copy records of all instrument calibrations are maintained in the laboratory. Corrective actions to remedy an out-of-control situation are documented.

An injection log book is maintained for the ICP-MS and includes the method used for quantitation, the sequence of injections which details the analysis of blanks, standards, and samples, the data file storage reference for each injection, the instrument identification and the analyst identification.

Whenever possible, the laboratory will use NIST-traceable standards for the project. Records of standard preparations are maintained. A standard log book is used to document the preparation of standards and provide a means to trace each solution to the starting material. Each entry is dated and signed by the preparer. Standards are labeled by reference to standard log, identification of material, nominal concentration, preparation and expiration dates, and identification of preparer. Stock and working solutions are prepared fresh as required by their stability and are checked regularly for signs of deterioration. Stock and working standards are disposed of properly if signs of deterioration are evident, or on their expiration date, whichever occurs first.

B8. Inspection/Acceptance of Supplies and Consumables

Supplies and consumables include reagents, standards, 96 well microtiter plates (with filtration apparatus and without), pipette tips, high purity Argon gas, paper supplies, computer supplies, and instrument parts. Items from each lot of supplies or consumables that are used in large quantities, such as microtiter plates and pipette tips, are tested prior to first use to assure that they are free of contaminants of concern and that the quality does not affect the outcome of the project. Standards are checked prior to first use by comparing the quality or performance of the new batch or lot against existing materials. Concentrations must agree to within 10%. Reagents and Argon gas quality are monitored by the appearance and acceptability of lab procedural blanks. Other supplies and consumables are visually inspected for cleanliness, inadequate seals or wrapping, and expiration dates.

The laboratory's Research Scientist and Inorganic Chemistry Unit Supervisor are responsible to assure that supplies and consumables are of acceptable quality. If non-conformances are encountered, the analyst and Inorganic Chemistry Unit Supervisor must stop the analysis, obtain, and verify the quality of new supplies prior to resuming sample analysis.

B9. Non-direct Measurements

There are no data required from non-measurement sources for the implementation of this project.

B10. Data Management

All laboratory personnel involved in the project will use approved data forms and bound log books to record the findings of observations or to document their work. The forms and log books will be signed and dated. Before final results are released to the Principal Investigator, the data will be checked by an equally competent person. Changes to documentation must be dated and initialed. All files of data, whether paper or computer-acquired/generated shall be secured.

Samples are tracked from receipt to disposal using a laboratory information management system (LIMS). Results of sample analyses, quality control determinations, and any data qualifiers that describe data that do not meet the acceptance criteria established in the SOP are entered into LIMS. In addition, the data of the ICP-MS and most of the laboratory's other analytical instrumentation are networked and transferred electronically to the LIMS. Changes to the LIMS data are backed up each evening and the entire database is backed up in full each week, each month, and each year. Instrument data on the network are backed up on the same schedule. Backup tapes are kept in secure storage offsite.

Data are transformed from individual point values into related values in accordance with the equations in the MDH SOP for total mercury in blood spots.

Standard laboratory record-keeping procedures, document control systems, and data storage and retrieval can be found in MDH Laboratory QAM as follows:

1. Section 6.0 Information Technology.
2. Section 8.0 Sample Custody, Handling, and Tracking.
3. Section 14.0 Data Reduction, Verification, Validation, and Reporting

Data Interpretation and Reporting

The study will consist of approximately 1500 subjects. The data collected will be expressed as a concentration of total mercury in blood (ug/L). The data will be analyzed using geometric means. The distribution of the mercury levels in the basin will be characterized using means, standard deviations, and percentiles. The overall mean for the basin sample will be compared to the data on mercury levels in the general US population of young children and women of childbearing age. The national data will be obtained from the 2003-2004 National Health and Nutrition Examination Survey (NHANES) survey. The comparisons will be done using t-test comparisons. Analysis accounting for seasonality will be limited because of the small number of births in the basin per month and overall sample size.

Data will be reported to GLNPO as described in other sections of this QAPP. In addition, in cooperation with the GLNPO Project Officer we will disseminate the data and the results of our interpretation in reports, presentations and communications with the public. Local, state and tribal public health and health care providers will be invited to participate in the design and implementation of the communication of results and outreach.

SECTION C. ASSESSMENT AND OVERSIGHT

C1. Assessment and Response Actions

Assessments planned for the life of the project include surveillance, peer review, performance evaluation, quality system audits, and data quality assessments.

Surveillance

Surveillance will consist of frequent monitoring of the status of the project and an analysis of records as a check that the requirements of the project are being fulfilled. The MDH laboratory QA Officer will conduct weekly surveillance of the data produced for the project. All planned surveillance activities will be documented. If unplanned deviations from established protocols are determined during the course of surveillance activities, the Principal Investigator and the MDH Environmental Laboratory Manager will be notified in writing. The laboratory's Research Scientist, the Inorganic Chemistry Unit Supervisor, and the laboratory QA Officer are responsible to locate the source of the problem, and take corrective actions prior to resuming sample analysis.

The MDH Environmental Laboratory Manager has the authority to stop work on the project should the identified deviations, in her view, prevent the data from being judged as valid.

Peer Review

Peer review will consist of reviewing the results of the data by someone equally competent as the analyst performing the work. Documents will be reviewed for technical adequacy, accuracy, compliance with the established protocols and procedures, and editorial quality. The MDH laboratory Inorganic Chemistry Unit Supervisor and the MDH laboratory QA Officer will conduct peer review in an on-going manner over the course of the project. All peer review activities will be documented.

Proficiency Testing

The laboratory will seek out and enroll in any proficiency testing studies that may come available during the course of the project. There are no proficiency testing samples that are specific to this QAPP; however, the laboratory will continue to participate in proficiency testing studies of total mercury in whole blood. Throughout the course of the project, challenge samples of mercury on dried blood spots will be presented irregularly as routine samples to the Research Scientist. The Principal Investigator will evaluate the data resulting from the challenge samples.

Quality System Audits

Independent assessments of the laboratory's ability to generate data of a quality sufficient to meet the needs of the projects that the laboratory is involved with are conducted regularly by auditors that are functionally independent from the laboratory, in particular by auditors from EPA and the Centers for Medicare and Medicaid Services (CMS), which administers the CLIA inspections. An audit by a CLIA inspector is planned for fall

2008 and should include a review of the SOP for total mercury in dried blood spots. The MDH laboratory QA Officer conducts internal laboratory and management system audits in an on-going manner over the course of a year. All internal audit activities are documented.

Data Quality Assessments

After all the data have been collected, the Inorganic Chemistry Unit Supervisor and the MDH laboratory QA Officer will assess whether the data were accumulated, transferred, reduced, calculated, summarized, and reported correctly. All data that meet the MQOs listed in A1 Table 2 will be considered acceptable for project decision making.

All planned assessments and audits will be completed and documented.

Response Actions

Corrective actions will be taken when errors, non-conformances, or out-of-control situations exist. All potentially affected data must be thoroughly reviewed for acceptance or rejection.

C2. Reports to Management

The PI will provide semi-annual progress reports to the EPA Project Officer summarizing all progress to date, results of any performance or internal audits, interim data quality assessments and any notable lapses in quality assurance and plans for addressing these problems. A Final Project Report will be provided to the EPA Project Officer at the end of the project that includes all data and data interpretation.

SECTION D. DATA VALIDATION AND USABILITY

D1. Data Review, Verification and Validation

All data meeting the Measurement Quality Objectives (Table 2) will be considered acceptable and usable by the project. Data having any QA qualifiers will be carefully examined to determine if the qualifier invalidates the data, or whether the data are still judged acceptable despite the QA qualifier, based on professional judgment of the PI.

D2. Verification and Validation Methods

The PI will review all data generated. The use of laboratory spikes, check samples, and performance standards serves to verify that the systems and procedures are working correctly and to validate the results of the project.

D3. Reconciliation with User Requirements

The MQOs have been designed to provide data such that the DQOs of the project will be met.

ACKNOWLEDGMENTS

The Principal Investigator would like to acknowledge the contributors to this QAPP and its appendices for their assistance and expertise. Contributors include: Jeffrey Brenner, Betsy Edhlund, Myron Falken, Louise Liao, Paula Lindgren, Mark McCann, Pamela Shubat, Suzanne Skorich, Carrie Wolf, and Zheng Yang. They are employees of the Minnesota Department of Health and will be vital to the implementation of this project. The Principal Investigator would also like to acknowledge Kevin Cavanagh and Gary Hoffman in the newborn screening programs in Michigan and Wisconsin, respectively, for their willingness to participate in this collaborative study.

REFERENCES

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ESRI. 2005. ESRI® Data & Maps. ESRI, Redlands, California, USA

NRRI. 1999. Watershed Boundary for Lake Superior. Natural Resource Research Institute (NRRI), Duluth, Minnesota. <http://www.nrri.umn.edu/lsgis/index.htm>

APPENDICES

- Appendix A Method of Punching Dried Blood Spots on Filter Paper for Mercury Biomonitoring in Newborns in the Lake Superior Basin and Other Minnesota Locations
- Appendix B Procedure for the Determination of: Mercury in Dried Blood Spots by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)
- Appendix C Quality Assurance Manual for the Environmental Laboratory Testing Units Public Health Laboratory Division Minnesota Department of Health
- Appendix D Organizational Charts



**METHOD OF PUNCHING DRIED BLOOD SPOTS ON FILTER PAPER FOR
MERCURY BIOMONITORING IN NEWBORNS IN THE LAKE SUPERIOR BASIN
AND OTHER MINNESOTA LOCATIONS**

APPROVAL

Written By: Carrie Wolf Date: 02-28-2008

Carrie Wolf, Environmental Analyst 2

Revised By: _____ Date: 09-15-2009

Carrie Wolf, Environmental Analyst 3

Approved By: _____ Date: 09-15-2009

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Approved By: _____ Date: 09-18-2009

Mark T. McCann, Newborn Screening Laboratory Manager

Approved By: _____ Date: 09-22-2009

Sharon Pendergrass, QC/QA Officer

Approved By: _____ Date: 09-23-2009

Joanne M. Bartkus, Public Health Laboratory Director

Newborn Screening Program, Public Health Laboratory

METHOD OF PUNCHING DRIED BLOOD SPOTS ON FILTER PAPER FOR MERCURY BIOMONITORING IN NEWBORNS IN THE LAKE SUPERIOR BASIN AND OTHER MINNESOTA LOCATIONS

1.0 SCOPE AND APPLICATION

- 1.1 Employees acting on behalf of the Newborn Screening Programs (NBS) at the Minnesota Department of Health (MDH), the Wisconsin State Laboratory of Hygiene (WSLH), and the Michigan Department of Community Health (MDCH) will collect blood spots to determine the mercury levels from newborns in the Lake Superior basin.

2.0 SUMMARY OF METHOD

- 2.1 Babies born to mothers who live in the Lake Superior basin in the states of Minnesota, Wisconsin, and Michigan will have their newborn screening cards tested for mercury levels. Before any specimens from Minnesota can be used, MDH will receive permission from a parent for use of their child's residual dried blood spot in this study. Four 3-mm (1/8-inch) discs will be punched from the residual dried blood spots corresponding to each selected newborn into a plate and sealed with strip caps for transportation. The validity of the specimen is determined before the spot is punched. Four 3-mm discs of blank filter paper will also be punched from each selected newborn's specimen card into the plate. On every twentieth specimen, four additional 3-mm blood spot discs and blank filter paper discs will be punched for quality control determinations. Specimens from Wisconsin and Michigan will be mailed to MDH for analysis.

3.0 SAFETY

- 3.1 Analysts who work in the laboratory should follow each state's laboratory safety policy. If there are any questions as to what the policies are, contact the newborn screening supervisor from each state.
- 3.2 Safety glasses must be worn by analysts when handling chemicals and reagents. Lab coats and other protective clothing, such as gloves, should be worn by analysts when appropriate.

4.0 EQUIPMENT AND SUPPLIES

- 4.1 Wallac MultipuncherTM (or equivalent), plus computer and printer, available by PerkinElmer, Catalog # 1296-081.
- 4.2 Eppendorf twin.tec PCR skirted 96 well microtitre plates, Catalog #951020401 (Supplied by MDH).

- 4.3 Eppendorf Cap Strips, Catalog #951023035 (Supplied by MDH).
- 4.4 VWR Plastic Bags 9x12 inches, Catalog #WL52870 (Supplied by MDH).
- 4.5 Freezers capable of constant temperatures from 0 to -30°C.

5.0 SPECIMEN COLLECTION AND STORAGE

- 5.1 MDH, WSLH, and MDCH will select blood spots for analysis with the understanding that the blood spots have been collected using the following guidelines.
 - 5.1.1 Blood should be collected on filter cards by heel stick from newborn infants 24 – 48 hours old. (In rare circumstances, the first collection can occur up to a few weeks after birth. For this mercury biomonitoring study, specimens are deemed to be acceptable if they are collected before the infant is ten days of age.)
 - 5.1.2 For complete collection instructions, refer to the publication of the Clinical and Laboratory Standards Institute (CLSI): Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard-Fifth Edition (LA4-A5, Vol. 27, No. 20).
 - 5.1.3 Specimens are air dried for at least 3-4 hours and mailed or couriered to each state's Newborn Screening Laboratory.
 - 5.1.4 The specimen cards are stored per individual laboratory protocol.
- 5.2 After the specimens have been punched into the plates, the plates should be stored in a freezer, maintained at $-20 \pm 5^{\circ}\text{C}$, until they are shipped or hand delivered to the Environmental Accessioning area of the MDH Public Health Laboratory. The Inorganic Chemistry Unit at MDH will store the specimens in a freezer, maintained at $-20 \pm 5^{\circ}\text{C}$, until analysis.

6.0 PROCEDURE

- 6.1 Equipment and Supplies: MDH will supply all participating facilities with plates and strip caps into which the facilities will punch the dried bloods spots and filter blanks using a Wallac MultipuncherTM. This is to ensure minimum variation between the three state newborn screening programs. Each state will follow their own maintenance protocols for cleaning of the Wallac MultipuncherTM.
- 6.2 Specimen Selection: When newborn screening testing is fully completed, the specimens with ZIP codes from the Lake Superior Basin will be pulled for use in this study. All repeat specimens from a single newborn should be excluded from the study. Specimens from transfused babies should also be excluded. No specimens will be considered for use in the study if the specimen collection date is greater than 10 days of age.
 - 6.2.1 ZIP code information is not included on the Wisconsin NBS cards. Wisconsin Vital Records will provide the Wisconsin NBS facility with a list of specimens

with ZIP codes listed in Attachment 9.1, “ZIP Code Lists for Michigan, Minnesota, and Wisconsin”.

- 6.2.2 Only the MDH Newborn Screening Program will need written, informed consent before they can release a residual specimen for use in this study. After NBS work is complete, a query in the Laboratory Information Management System (LIMS) will be used to provide a weekly list of specimens that meet the following criteria:
- Mother’s residence ZIP code, listed on the specimen card, is in the Lake Superior Basin per the list of ZIP codes in Attachment 9.1, “ZIP Code Lists for Michigan, Minnesota, and Wisconsin”.
 - No specimens included where families have directed that their child’s blood spot card be destroyed.
 - No transfused sample specimens or babies with more adult hemoglobin than fetal hemoglobin will be included.
 - No repeat samples included (when a hospital or other responsible party has collected specimens from a single infant on more than one day and submitted separate cards, only the specimens collected on the earliest date should be used).
 - Specimens included only when there are fewer than 10 days between the date of birth and the date of collection.
 - No risk factors have been checked on the specimen card including: sick baby, deceased sibling, congenital anomalies, and maternal pregnancy complications.
 - All of the newborn screening results are normal.
 - All babies known to have been admitted to a Neonatal Intensive Care Unit (NICU) or with a birth weight under 2000 grams will be excluded from the group.
 - All deceased babies will be excluded from the study.
 - Specimens from St. Mary’s hospital in Duluth, MN taken less than 24 hours from birth will be excluded from the study.

Informed Consent: The list of specimens meeting the criteria in 6.2 and 6.3 will be checked to ensure there are no repeat records or babies known to be deceased. A unique Informed Consent Identification Number and ZIP code cluster will be assigned to each record in the list. The Informed Consent Identification Number will be used for tracking the informed consent process but will not be carried forward once consent is achieved. When informed consent is received a copy of the signed form will be mailed back to the participant. The NBS lab will retain the original in the records storage room in the administrative area of the Public Health Laboratory. Once written consent is received, the specimen can be used in the study. Local Public Health will attempt to obtain informed consent from the families they visit as part of their normal new baby visits and will mail signed informed consent forms to MDH mercury study staff.

- 6.3 Determination of Satisfactory Specimen: Attachment 9.2 contains a copy of the CLSI

Simple Spot Check, which should be followed to identify satisfactory specimens. If the specimen is determined as unsatisfactory or if there is not an adequate amount of blood, the specimen should be excluded from the study. If there is enough blood for the study, but there will be insufficient blood left over for the newborn screening laboratory, the specimen should be excluded from the study. All repeat specimens should also be excluded from the study. MDH will not request informed consent on unsatisfactory specimens.

6.4 Specimen Punching: Punch four discs of dried blood into two wells, so there are two blood spot discs per well for each newborn screening card included in the study. Also punch four discs of blank filter paper into two wells, so there are two blank filter paper discs in each well for each of the newborn screening cards. Punch an additional four discs of dried blood and blank filter paper on every twentieth NBS card that is eligible for inclusion in the study.

- Punch specimens into microtiter plates and seal with strip caps (both supplied by MDH for the mercury biomonitoring project).
- Use the Wallac MultipuncherTM to punch the specimens into the plates.
- Avoid ink as much as possible while punching the blood spots and filter paper blanks.
- Punch the dried blood spot discs from as many dried blood spots on the card as possible to account for any variation among different blood spots on the newborn screening card. For example, MDH only has three available dried blood spots on their newborn screening card. Two of the dried blood spots will have a single punch taken out of it and one blood spot will have two punches taken out of it for a total of four dried blood spot discs.

6.4.1 A total of twenty specimens can be punched into one plate. Create a punching gridsheet for the specimens to be punched (See Attachment 9.5 for a copy of this form). Each well on the punching gridsheet will contain the Study ID Number (e.g. MN-0001) and indicate if there are dried blood spots or filter blanks in the wells. The following wells are to remain empty: E11, F11, G11, H11, A12, B12, C12, D12, E12, F12, G12, & H12. MDH will send a punching gridsheet template to be used by all participants in the study and a copy of the form is provided in Attachment 9.5. Number the plates with a permanent marker and write the same number on the corresponding gridsheet.

6.4.2 When punching the first specimen, two blood spot discs will be punched into well A1 and two blood spot discs will be punched into well A2. Punch two blank filter paper discs into wells A3 and A4 from the same specimen card. Continue this pattern for all of the twenty specimens for the plate. The twentieth specimen will have an additional four dried blood spots and filter paper blanks punched. Wells E10, F10, G10, & H10 will contain two dried blood spot discs and wells A11, B11, C11, & D11 will contain two filter paper blank discs. The punching gridsheet should match attachments 9.3 and 9.4 “Infant Descriptor for Mercury Dried Blood Specimens” and the “Chain of Custody for Mercury Dried Blood Specimens.” Cover all wells on the plate with strip caps when the punching has been completed.

6.4.3 Store the plates in a -20°C freezer, maintained at $\pm 5^{\circ}\text{C}$ until the specimens can be

mailed or delivered to MDH Environmental Accessioning area.

- 6.5 Mailing/Delivery of Specimens: To ensure anonymity a minimum of five specimens per sex, per ZIP code cluster, per month is needed before the samples are shipped to the MDH. If these minimum criteria are not met, the facility should contact the Principal Investigator, Pat McCann (patricia.mccann@state.mn.us or 651/201-4915), for further instructions. The plates containing specimens must be accompanied by a completed Blood Specimen Criteria Checklist, chain of custody form, and punching gridsheet when mailed to MDH. A copy of the chain of custody form is provided in Attachment 9.4, “Chain of Custody for Mercury Dried Blood Specimens.” The chain of custody form is a list of specimens that is to be shipped to MDH. The plates will be placed in large reclosable bags supplied by MDH along with the chain of custody form. The specimens should be shipped with ice packs, so the temperature is maintained at $<10^{\circ}\text{C}$ throughout the shipment. The opening of the iced compartment should be completely sealed with packing tape and signed and dated across the tape to assure integrity of the contents during transit. The specimens should be mailed to the address provided on the chain of custody form using the pre-paid, addressed Fed Ex labels supplied by MDH so that delivery is made to the Environmental Accessioning area of the MDH Public Health Laboratory. MDH will hand deliver their monthly batches. The specimens will be assigned and labeled with a MDH accession number and entered into the Laboratory Information Management System (LIMS) by Environmental Accessioning staff and delivered to the Inorganic Chemistry Unit. The MDH accession number will be linked to the Study ID Number on the “Chain of Custody for Mercury Dried Blood Specimens.” An authorized representative of the Inorganic Chemistry Unit will place the specimens in a secure -20°C freezer, maintained at $\pm 5^{\circ}\text{C}$.
- 6.6 Notification of Shipped Specimens: After each facility mails or delivers the specimens to the MDH Environmental Accessioning area, an authorized staff member must email the completed form, “Infant Descriptors for Mercury Dried Blood Specimens,” to patricia.mccann@state.mn.us as a password-protected document and not to the Inorganic Chemistry Laboratory personnel. (See Attachment 9.3 for a copy of this form).

7.0 QUALITY CONTROL

- 7.1 Reference Sample Specimens: Periodically throughout the study, the MDH Environmental Laboratory Quality Assurance Officer will supply reference sample specimen cards with blood spots of known mercury concentration values to the participating laboratories. These specimens should be treated like normal specimens for this study and be collected in accordance with the schedule provided with the reference sample specimens and using the above procedure for specimen punching (Section 6.4). Do not choose the reference sample as the twentieth specimen that will have additional punches taken out of it. Also vary the location of the reference sample on the plate. The laboratory punching the reference samples shall assign a Study ID Number to the reference sample discs and the blank filter paper discs and fill out attachments 9.3 and 9.4, “Infant Descriptors for Mercury Dried Blood Specimens”, and “Chain of Custody for

Mercury Dried Blood Specimens.” The three state newborn screening programs will submit these reference sample specimens along with batches of the punched discs from residual newborn specimens. These reference sample specimens will be single-blinded to the Inorganic Chemistry analyst. When completing the “Infant Descriptors for Mercury Dried Blood Specimens” form, indicate that the blood spots are reference sample specimens by writing “REF” in the last column (See Attachment 9.3). This form must be emailed to patricia.mccann@state.mn.us as a password-protected document and not to the Inorganic Chemistry Laboratory personnel. (See Attachment 9.3 for a copy of this form.)

8.0 REFERENCES

- 8.1 CLSI. *Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard-Fifth Edition*. CLSI document LA4-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2007

9.0 ATTACHMENT

9.1 ZIP Code Lists for Michigan, Minnesota, and Wisconsin

ZIP CODE	PO NAME	STATE	Zip Code Cluster
49715	BRIMLEY	MI	non-urban
49724	DAFTER	MI	non-urban
49728	ECKERMAN	MI	non-urban
49748	HULBERT	MI	non-urban
49752	KINROSS	MI	non-urban
49762	NAUBINWAY	MI	non-urban
49768	PARADISE	MI	non-urban
49780	RUDYARD	MI	non-urban
49783	SAULT SAINTE MARIE	MI	East UP Urban
49784	KINCHELOE	MI	East UP Urban
49785	KINCHELOE	MI	East UP Urban
49788	KINCHELOE	MI	East UP Urban
49790	STRONGS	MI	non-urban
49793	TROUT LAKE	MI	non-urban
49805	ALLOUEZ	MI	West UP Urban
49806	AU TRAIN	MI	non-urban
49808	BIG BAY	MI	non-urban
49814	CHAMPION	MI	non-urban
49816	CHATHAM	MI	non-urban
49822	DEERTON	MI	non-urban
49825	EBEN JUNCTION	MI	non-urban
49826	RUMELY	MI	non-urban
49839	GRAND MARAIS	MI	non-urban
49841	GWINN	MI	non-urban
49849	ISHPEMING	MI	East UP Urban
49853	MC MILLAN	MI	non-urban
49855	MARQUETTE	MI	East UP Urban
49861	MICHIGAMME	MI	non-urban
49862	MUNISING	MI	non-urban
49865	NATIONAL MINE	MI	East UP Urban
49866	NEGAUNEE	MI	non-urban
49868	NEWBERRY	MI	non-urban
49871	PALMER	MI	non-urban
49883	SENEY	MI	non-urban
49884	SHINGLETON	MI	non-urban
49885	SKANDIA	MI	non-urban
49891	TRENARY	MI	non-urban
49895	WETMORE	MI	non-urban
49901	AHMEEK	MI	West UP Urban
49905	ATLANTIC MINE	MI	non-urban
49908	BARAGA	MI	non-urban
49910	BERGLAND	MI	non-urban
49911	BESSEMER	MI	non-urban
49912	BRUCE CROSSING	MI	non-urban
49913	CALUMET	MI	West UP Urban
49915	CASPIAN	MI	non-urban
49916	CHASSELL	MI	non-urban
49917	COPPER CITY	MI	West UP Urban
49918	COPPER HARBOR	MI	non-urban
49919	COVINGTON	MI	non-urban
49921	DODGEVILLE	MI	non-urban
49922	DOLLAR BAY	MI	West UP Urban
49925	EWEN	MI	non-urban
49929	GREENLAND	MI	non-urban
49930	HANCOCK	MI	West UP Urban
49931	HOUGHTON	MI	West UP Urban
49934	HUBBELL	MI	non-urban
49935	IRON RIVER	MI	non-urban
49938	IRONWOOD	MI	West UP Urban
49945	LAKE LINDEN	MI	non-urban
49946	LANSE	MI	non-urban
49947	MARENISCO	MI	non-urban
49948	MASS CITY	MI	non-urban
49950	MOHAWK	MI	non-urban
49952	NISULA	MI	non-urban
49953	ONTONAGON	MI	non-urban
49955	PAINESDALE	MI	non-urban
49958	PELKIE	MI	non-urban
49959	RAMSAY	MI	non-urban
49960	ROCKLAND	MI	non-urban
49961	SIDNAW	MI	non-urban
49962	SKANEE	MI	non-urban
49963	SOUTH RANGE	MI	non-urban
49964	STAMBAUGH	MI	non-urban
49965	TOIVOLA	MI	non-urban
49967	TROUT CREEK	MI	non-urban
49968	WAKEFIELD	MI	non-urban
49969	WATERSMEET	MI	non-urban
49970	WATTON	MI	non-urban
49971	WHITE PINE	MI	non-urban

ZIP CODE	PO_NAME	STATE	Zip Code Cluster
55601	BEAVER BAY	MN	non-urban
55602	BRIMSON	MN	non-urban
55603	FINLAND	MN	non-urban
55604	GRAND MARAIS	MN	non-urban
55605	GRAND PORTAGE	MN	non-urban
55606	HOVLAND	MN	non-urban
55607	ISABELLA	MN	non-urban
55609	KNIFE RIVER	MN	non-urban
55612	LUTSEN	MN	non-urban
55613	SCHROEDER	MN	non-urban
55614	SILVER BAY	MN	non-urban
55615	TOFTE	MN	non-urban
55616	TWO HARBORS	MN	non-urban
55701	ADOLPH	MN	non-urban
55702	ALBORN	MN	non-urban
55705	AURORA	MN	non-urban
55706	BABBITT	MN	non-urban
55707	BARNUM	MN	non-urban
55708	BIWABIK	MN	non-urban
55710	BRITT	MN	non-urban
55711	BROOKSTON	MN	non-urban
55713	BUHL	MN	urban
55717	CANYON	MN	non-urban
55718	CARLTON	MN	non-urban
55719	CHISHOLM	MN	urban
55720	CLOQUET	MN	non-urban
55724	COTTON	MN	non-urban
55726	CROMWELL	MN	non-urban
55732	EMBARRASS	MN	non-urban
55733	ESKO	MN	non-urban
55734	EVELETH	MN	non-urban
55736	FLOODWOOD	MN	non-urban
55738	FORBES	MN	non-urban
55741	GILBERT	MN	non-urban
55742	GOODLAND	MN	non-urban
55746	HIBBING	MN	urban
55747	HIBBING	MN	urban
55749	HOLYOKE	MN	non-urban
55750	HOYT LAKES	MN	non-urban
55751	IRON	MN	non-urban
55752	JACOBSON	MN	non-urban
55756	KERRICK	MN	non-urban
55758	KINNEY	MN	urban
55763	MAKINEN	MN	non-urban
55765	MEADOWLANDS	MN	non-urban
55766	MELRUDE	MN	non-urban
55767	MOOSE LAKE	MN	non-urban
55768	MOUNTAIN IRON	MN	non-urban
55775	PENGILLY	MN	non-urban
55777	VIRGINIA	MN	urban
55779	SAGINAW	MN	non-urban
55780	SAWYER	MN	non-urban
55782	SOUDAN	MN	non-urban
55784	SWAN RIVER	MN	non-urban
55787	TAMARACK	MN	non-urban
55790	TOWER	MN	non-urban
55791	TWIG	MN	non-urban
55792	VIRGINIA	MN	urban
55793	WARBA	MN	non-urban
55797	WRENSHALL	MN	non-urban
55801	DULUTH	MN	urban
55802	DULUTH	MN	urban
55803	DULUTH	MN	non-urban
55804	DULUTH	MN	urban
55805	DULUTH	MN	urban
55806	DULUTH	MN	urban
55807	DULUTH	MN	urban
55808	DULUTH	MN	urban
55810	DULUTH	MN	non-urban
55811	DULUTH	MN	urban
55812	DULUTH	MN	urban
55814	DULUTH	MN	urban
55815	DULUTH	MN	urban
55816	DULUTH	MN	urban

ZIP CODE	PO_NAME	STATE	Zip Code Cluster
54517	CLAM LAKE	WI	WI
54519	CONOVER	WI	WI
54525	GILE	WI	WI
54527	GLIDDEN	WI	WI
54534	HURLEY	WI	WI
54536	IRON BELT	WI	WI
54540	LAND O LAKES	WI	WI
54546	MELLEN	WI	WI
54547	MERCER	WI	WI
54550	MONTREAL	WI	WI
54557	PRESQUE ISLE	WI	WI
54559	SAXON	WI	WI
54565	UPSON	WI	WI
54806	ASHLAND	WI	WI
54814	BAYFIELD	WI	WI
54816	BENOIT	WI	WI
54820	BRULE	WI	WI
54821	CABLE	WI	WI
54827	CORNUCOPIA	WI	WI
54832	DRUMMOND	WI	WI
54836	FOXBORO	WI	WI
54838	GORDON	WI	WI
54839	GRAND VIEW	WI	WI
54842	HAWTHORNE	WI	WI
54844	HERBSTER	WI	WI
54846	HIGH BRIDGE	WI	WI
54847	IRON RIVER	WI	WI
54849	LAKE NEBAGAMON	WI	WI
54850	LA POINTE	WI	WI
54854	MAPLE	WI	WI
54855	MARENGO	WI	WI
54856	MASON	WI	WI
54861	ODANAH	WI	WI
54864	POPLAR	WI	WI
54865	PORT WING	WI	WI
54873	SOLON SPRINGS	WI	WI
54874	SOUTH RANGE	WI	WI
54880	SUPERIOR	WI	WI
54891	WASHBURN	WI	WI

9.2 CLSI Simple Spot Check

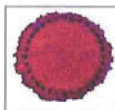
Volume 27

LA4-A5

Appendix B. Simple Spot Check (Modified and reprinted with permission from Whatman, Inc.)

Simple Spot Check

Valid specimen:



Allow a sufficient quantity of blood to soak through to completely fill the preprinted circle on the filter paper. Fill all required circles with blood. Do not layer successive drops of blood or apply blood more than once in the same collection circle. Avoid touching or smearing spots.

Invalid specimen:

Possible causes:



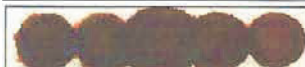
1. Specimen quantity insufficient for testing.



2. Specimen appears scratched or abraded.



3. Specimen not dry before mailing.



4. Specimen appears supersaturated.



5. Specimen appears diluted, discolored or contaminated.



6. Specimen exhibits serum rings.



7. Specimen appears clotted or layered.



8. No blood.

- Removing filter paper before blood has completely filled circle or before blood has soaked through to second side.
- Applying blood to filter paper with a capillary tube.
- Allowing filter paper to come into contact with gloved or ungloved hands or substances such as hand lotion or powder, either before or after blood specimen collection.

- Applying blood with a capillary tube or other device.

- Mailing specimen before drying for a minimum of three hours.

- Applying excess blood to filter paper, usually with a device.
- Applying blood to both sides of filter paper.

- Squeezing or "milking" of area surrounding the puncture site.
- Allowing filter paper to come into contact with gloved or ungloved hands or substances such as alcohol, formula, antiseptic solutions, water, hand lotion or powder, etc., either before or after blood specimen collection.
- Exposing blood spots to direct heat.

- Not allowing alcohol at the puncture site to air dry before making skin puncture.
- Allowing filter paper to come into contact with alcohol, hand lotion, etc.
- Squeezing area surrounding puncture site excessively.
- Drying specimen improperly.
- Applying blood to filter paper with a capillary tube.

- Touching the same circle on filter paper to blood drop several times.
- Filling circle on both sides of filter paper.

- Failure to obtain blood specimen.

9.3 Infant Descriptors for Mercury Dried Blood Specimens

		Minnesota Department of Health Environmental Health Division 601 Robert St. N. Saint Paul, MN, 55155-2531		Infant Descriptors for Mercury Dried Blood Specimens
Submitted By: Newborn Screening Laboratory Minnesota Public Health Laboratory, Minnesota Department of Health 601 Robert St. N., St. Paul, MN 55155-2531				
Study ID	ZIP Code Cluster	Birth Year	Birth Period Month	Sex M: Male F:Female Ref: Reference Standard
MN-0001				
MN-0002				
MN-0003				
MN-0004				
MN-0005				
MN-0006				
MN-0007				
MN-0008				
MN-0009				
MN-0010				
MN-0011				
MN-0012				
MN-0013				
MN-0014				
MN-0015				
MN-0016				
MN-0017				
MN-0018				
MN-0019				
MN-0020				
MN-0021				
MN-0022				
MN-0023				
MN-0024				
MN-0025				
MN-0026				
MN-0027				
MN-0028				
MN-0029				
MN-0030				
MN-0031				
MN-0032				
MN-0033				
MN-0034				
MN-0035				
MN-0036				
MN-0037				
MN-0038				
MN-0039				
MN-0040				
This information should be only viewed by designated MDH staff.				



Minnesota Department of Health
Environmental Health Division
601 Robert St. N. Saint Paul, MN, 55155-2531

Infant Descriptors
for Mercury Dried Blood Specimens

Submitted By:
Newborn Screening Laboratory
Wisconsin State Laboratory of Hygiene
465 Henry Mall, Madison, WI 53706

Study ID Number	ZIP Code Cluster	Birth Year	Birth Period Month	Sex M: Male F:Female Ref: Reference Standard
WI-0001	WI			
WI-0002	WI			
WI-0003	WI			
WI-0004	WI			
WI-0005	WI			
WI-0006	WI			
WI-0007	WI			
WI-0008	WI			
WI-0009	WI			
WI-0010	WI			
WI-0011	WI			
WI-0012	WI			
WI-0013	WI			
WI-0014	WI			
WI-0015	WI			
WI-0016	WI			
WI-0017	WI			
WI-0018	WI			
WI-0019	WI			
WI-0020	WI			
WI-0021	WI			
WI-0022	WI			
WI-0023	WI			
WI-0024	WI			
WI-0025	WI			
WI-0026	WI			
WI-0027	WI			
WI-0028	WI			
WI-0029	WI			
WI-0030	WI			
WI-0031	WI			
WI-0032	WI			
WI-0033	WI			
WI-0034	WI			
WI-0035	WI			
WI-0036	WI			
WI-0037	WI			
WI-0038	WI			
WI-0039	WI			
WI-0040	WI			

This information should be only viewed by designated MDH staff.



Minnesota Department of Health
Environmental Health Division
601 Robert St. N. Saint Paul, MN, 55155-2531

Infant Descriptors
for Mercury Dried Blood Specimens

Submitted By:

Newborn Screening Laboratory, Chemistry and Toxicology Division

Michigan Public Health Laboratory, Michigan Department of Community Health

3350 North Martin Luther King Jr. Blvd, Bldg 44, Lansing, MI 48909

Study ID Number	ZIP Code Cluster	Birth Year	Birth Period Month	Sex M: Male F:Female Ref: Reference Standard
MI-0001				
MI-0002				
MI-0003				
MI-0004				
MI-0005				
MI-0006				
MI-0007				
MI-0008				
MI-0009				
MI-0010				
MI-0011				
MI-0012				
MI-0013				
MI-0014				
MI-0015				
MI-0016				
MI-0017				
MI-0018				
MI-0019				
MI-0020				
MI-0021				
MI-0022				
MI-0023				
MI-0024				
MI-0025				
MI-0026				
MI-0027				
MI-0028				
MI-0029				
MI-0030				
MI-0031				
MI-0032				
MI-0033				
MI-0034				
MI-0035				
MI-0036				
MI-0037				
MI-0038				
MI-0039				
MI-0040				

This information should be only viewed by designated MDH staff.

9.4 Chain of Custody for Mercury Dried Blood Specimens

 Minnesota Department of Health Public Health Laboratory 601 Robert St. N. Saint Paul, MN, 55155-2531				page ____ of ____ Chain of Custody for Mercury Dried Blood Specimen
Submitted By: Newborn Screening Laboratory Minnesota Department of Health, Public Health Laboratory 601 Robert St. N. Saint Paul, MN, 55155-2531				
Dried Blood Spot Punched by (print):		Signature:		Date (mm/dd/yy):
Completed Check List on Back: ____ (Initial)				
Study ID Number	Residual Specimen Punch Date (dd/mm/yy)	Number of Dried Blood Punches (3mm)	Number of Blank Filter Punches (3mm)	MDH Accession Number (MDH Lab use only)
MN-0001				
MN-0002				
MN-0003				
MN-0004				
MN-0005				
MN-0006				
MN-0007				
MN-0008				
MN-0009				
MN-0010				
MN-0011				
MN-0012				
MN-0013				
MN-0014				
MN-0015				
MN-0016				
MN-0017				
MN-0018				
MN-0019				
MN-0020				
Relinquished by (print):		Signature:		Date (mm/dd/yy):
Signature acknowledges adherence to Method: Punching Dried Blood Spots on Filter Paper for Mercury Biomonitoring in Newborns in the Lake Superior Basin. Criteria indicated on back of chain of custody.				
MDH Use Only:				
Received by (print):		Signature:		Date (mm/dd/yy):



Minnesota Department of Health
Public Health Laboratory
601 Robert St. N. Saint Paul, MN, 55155-2531

page ____ of ____
Chain of Custody
for Mercury Dried Blood Specimen

Submitted By:
Newborn Screening Laboratory
Wisconsin State Laboratory of Hygiene
465 Henry Mall, Madison, WI 53706

Dried Blood Spot Punched by (print): Signature: Date (mm/dd/yy):

Completed Check List on Back: ____ (Initial)

Study ID Number	Residual Specimen Punch Date (dd/mm/yy)	Number of Dried Blood Punches (3mm)	Number of Blank Filter Punches (3mm)	MDH Accession Number (MDH Lab use only)
WI-0001				
WI-0002				
WI-0003				
WI-0004				
WI-0005				
WI-0006				
WI-0007				
WI-0008				
WI-0009				
WI-0010				
WI-0011				
WI-0012				
WI-0013				
WI-0014				
WI-0015				
WI-0016				
WI-0017				
WI-0018				
WI-0019				
WI-0020				

Relinquished by (print): Signature: Date (mm/dd/yy):

Signature acknowledges adherence to Method: Punching Dried Blood Spots on Filter Paper for Mercury Biomonitoring in Newborns in the Lake Superior Basin. Criteria indicated on back of chain of custody.

MDH Use Only:
Received by (print): Signature: Date (mm/dd/yy):



Minnesota Department of Health
Public Health Laboratory
601 Robert St. N. Saint Paul, MN, 55155-2531

page ____ of ____
Chain of Custody
for Mercury Dried Blood Specimen

Submitted By:
Newborn Screening Laboratory, Chemistry and Toxicology Division
Michigan Public Health Laboratory, Michigan Department of Community Health
3350 North Martin Luther King Jr. Blvd, Bldg 44, Lansing, MI 48906

Dried Blood Spot Punched by (print): _____ Signature: _____ Date (mm/dd/yy): _____

Completed Check List on Back: _____ (Initial)

Study ID Number	Residual Specimen Punch Date (dd/mm/yy)	Number of Dried Blood Punches (3mm)	Number of Blank Filter Punches (3mm)	MDH Accession Number (MDH Lab use only)
MI-0001				
MI-0002				
MI-0003				
MI-0004				
MI-0005				
MI-0006				
MI-0007				
MI-0008				
MI-0009				
MI-0010				
MI-0011				
MI-0012				
MI-0013				
MI-0014				
MI-0015				
MI-0016				
MI-0017				
MI-0018				
MI-0019				
MI-0020				

Relinquished by (print): _____ Signature: _____ Date (mm/dd/yy): _____

Signature acknowledges adherence to Method: Punching Dried Blood Spots on Filter Paper for Mercury Biomonitoring in Newborns in the Lake Superior Basin. Criteria indicated on back of chain of custody.

MDH Use Only:

Received by (print): _____ Signature: _____ Date (mm/dd/yy): _____



Minnesota Department of Health
Public Health Laboratory
601 Robert St. N. Saint Paul, MN, 55155-2531

Blood Specimen Criteria Checklist

- ☐ All tests for newborn screening are complete.
- ☐ Sufficient sample volume left over for newborn screening laboratory.
- ☐ Blood spots must meet the Simple Spot Check CLSI criteria before punching.
- ☐ Adequate amount of blood contained within blood spot to collect necessary number of discs.
- ☐ No repeat sample specimens.
- ☐ No specimens collected after transfusion.
- ☐ All samples collected should be less than 10 days between the date of birth and the date of collection.
- ☐ Every twentieth specimen, punch an additional four 3mm discs from the blood spot.
- ☐ When a reference sample is received from MDH, follow the attached schedule for sample collection (SOP section 7.1)
- ☐ All specimens stored in a -20°C freezer, before shipment to the Minnesota Laboratory.
- ☐ Make sure each monthly batch of samples contains at least 5 samples of each sex per zip code cluster before sending to the MDH metals lab.
- ☐ Chain of Custody form completed and shipped with samples to MDH.
- ☐ The specimens should be shipped with ice packs so the temperature is <10°C, package sealed with chain-of-custody tape.
- ☐ Electronic Infant Descriptor spreadsheet completed and e-mailed to patricia.mccann@state.mn.us

Comments: _____

Mercury Study

Plate 1

Date _____
Punched by _____

9.5 Mercury Study Gridsheets

	1	2	3	4	5	6	7	8	9	10	11	12
A	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Blanks	
	MN- 0001	MN- 0003	MN- 0005	MN- 0007	MN- 0009	MN- 0011	MN- 0013	MN- 0015	MN- 0017	MN- 0019	MN- 0020	
B	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Blanks	
	MN- 0001	MN- 0003	MN- 0005	MN- 0007	MN- 0009	MN- 0011	MN- 0013	MN- 0015	MN- 0017	MN- 0019	MN- 0020	
C	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	
	MN- 0001	MN- 0003	MN- 0005	MN- 0007	MN- 0009	MN- 0011	MN- 0013	MN- 0015	MN- 0017	MN- 0019	MN- 0020	
D	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	
	MN- 0001	MN- 0003	MN- 0005	MN- 0007	MN- 0009	MN- 0011	MN- 0013	MN- 0015	MN- 0017	MN- 0019	MN- 0020	
E	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots		
	MN- 0002	MN- 0004	MN- 0006	MN- 0008	MN- 0010	MN- 0012	MN- 0014	MN- 0016	MN- 0018	MN- 0020		
F	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots		
	MN- 0002	MN- 0004	MN- 0006	MN- 0008	MN- 0010	MN- 0012	MN- 0014	MN- 0016	MN- 0018	MN- 0020		
G	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Spots		
	MN- 0002	MN- 0004	MN- 0006	MN- 0008	MN- 0010	MN- 0012	MN- 0014	MN- 0016	MN- 0018	MN- 0020		
H	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Spots		
	MN- 0002	MN- 0004	MN- 0006	MN- 0008	MN- 0010	MN- 0012	MN- 0014	MN- 0016	MN- 0018	MN- 0020		

Mercury Study

Plate 1

Date _____
Punched by _____

	1	2	3	4	5	6	7	8	9	10	11	12
A	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Blanks	
	WI- 0001	WI- 0003	WI- 0005	WI- 0007	WI- 0009	WI- 0011	WI- 0013	WI- 0015	WI- 0017	WI- 0019	WI- 0020	
B	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Blanks	
	WI- 0001	WI- 0003	WI- 0005	WI- 0007	WI- 0009	WI- 0011	WI- 0013	WI- 0015	WI- 0017	WI- 0019	WI- 0020	
C	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	
	WI- 0001	WI- 0003	WI- 0005	WI- 0007	WI- 0009	WI- 0011	WI- 0013	WI- 0015	WI- 0017	WI- 0019	WI- 0020	
D	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	
	WI- 0001	WI- 0003	WI- 0005	WI- 0007	WI- 0009	WI- 0011	WI- 0013	WI- 0015	WI- 0017	WI- 0019	WI- 0020	
E	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots		
	WI- 0002	WI- 0004	WI- 0006	WI- 0008	WI- 0010	WI- 0012	WI- 0014	WI- 0016	WI- 0018	WI- 0020		
F	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots		
	WI- 0002	WI- 0004	WI- 0006	WI- 0008	WI- 0010	WI- 0012	WI- 0014	WI- 0016	WI- 0018	WI- 0020		
G	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Spots		
	WI- 0002	WI- 0004	WI- 0006	WI- 0008	WI- 0010	WI- 0012	WI- 0014	WI- 0016	WI- 0018	WI- 0020		
H	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Spots		
	WI- 0002	WI- 0004	WI- 0006	WI- 0008	WI- 0010	WI- 0012	WI- 0014	WI- 0016	WI- 0018	WI- 0020		

Mercury Study

Date _____
Punched by _____

Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Blanks	
	MI- 0001	MI- 0003	MI- 0005	MI- 0007	MI- 0009	MI- 0011	MI- 0013	MI- 0015	MI- 0017	MI- 0019	MI- 0020	
B	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Blanks	
	MI- 0001	MI- 0003	MI- 0005	MI- 0007	MI- 0009	MI- 0011	MI- 0013	MI- 0015	MI- 0017	MI- 0019	MI- 0020	
C	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	
	MI- 0001	MI- 0003	MI- 0005	MI- 0007	MI- 0009	MI- 0011	MI- 0013	MI- 0015	MI- 0017	MI- 0019	MI- 0020	
D	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	
	MI- 0001	MI- 0003	MI- 0005	MI- 0007	MI- 0009	MI- 0011	MI- 0013	MI- 0015	MI- 0017	MI- 0019	MI- 0020	
E	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots		
	MI- 0002	MI- 0004	MI- 0006	MI- 0008	MI- 0010	MI- 0012	MI- 0014	MI- 0016	MI- 0018	MI- 0020		
F	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots	Spots		
	MI- 0002	MI- 0004	MI- 0006	MI- 0008	MI- 0010	MI- 0012	MI- 0014	MI- 0016	MI- 0018	MI- 0020		
G	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Spots		
	MI- 0002	MI- 0004	MI- 0006	MI- 0008	MI- 0010	MI- 0012	MI- 0014	MI- 0016	MI- 0018	MI- 0020		
H	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks	Spots		
	MI- 0002	MI- 0004	MI- 0006	MI- 0008	MI- 0010	MI- 0012	MI- 0014	MI- 0016	MI- 0018	MI- 0020		

PROCEDURE FOR THE DETERMINATION OF:

**MERCURY IN DRIED BLOOD SPOTS
BY INDUCTIVELY COUPLED PLASMA MASS SPECTROSCOPY (ICP-MS): 748**

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9.0 PREPARATION OF REAGENTS, CALIBRATION STANDARDS, CONTROLS	9
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1.0 SCOPE AND APPLICATION

- 1.1 This method is applicable to laboratory screening of mercury in a dried blood spot formed on filter paper.
- 1.2 This method is applicable to total mercury (Hg) only.
- 1.3 This method describes the procedure to extract mercury from a dried blood spot with appropriate solvents, and the subsequent analysis by conventionally nebulized inductively coupled plasma mass spectrometry (ICP-MS).
- 1.4 The method should be used by analysts experienced in the use of ICP-MS technology, and handling of clinical specimens, or by laboratory assistants/technicians under the close supervision of experienced analysts.

2.0 SUMMARY OF METHOD

- 2.1 The intended samples are filter paper blood (dried blood spot) specimens initially collected for newborn screening testing.
- 2.2 Two 3-mm filter paper disks containing dried blood, punched from multiple blood spots on a filter paper card, are placed into a 96-well filter plate (or similar container) to which 0.15 ml of reagent water containing 0.05% 2-mercaptoethanol, 0.001% L-cysteine, 0.005% EDTA, 0.01% Triton X-100, and 10µg I⁻¹ of Iridium (internal standard) is added, followed by addition of 0.15ml of 2% hydrochloric acid. The covered filter plate is agitated for 30 minutes and stored overnight at room temperature. The filter plate is re-agitated for 20 minutes and the contents are filtered into a 96-well plate and placed on the ICP-MS auto sampler for analysis against a five- point aqueous standard calibration curve.
- 2.3 All the procedures should be conducted in a clean room facility.

3.0 DEFINITIONS

- 3.1 Definitions that are common to all units of the Laboratory appear in Section 2.0 of the Quality Assurance Manual.

4.0 INTERFERENCES

- 4.1 Method interferences may be caused by contaminants in reagent water, solvents, reagents, glassware, and other sample processing apparatus that can lead to discrete artifacts, elevated baselines or that may otherwise bias analyte response. All reagents and apparatus must be routinely demonstrated to be free from interferences by analyzing an Aqueous Blank (AB) immediately before any samples are analyzed and periodically throughout the run as needed.
- 4.2 For the determination of trace levels of mercury (Hg), contamination of the sample and loss of the analytes of interest are of prime consideration. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment from dust or other particulate matter. A clean laboratory work area, such as clean room facility, designed for trace element sample handling must be used. Standards, samples and blanks should be exposed to the laboratory environment as little as possible. The use of preparation blanks and spikes should be used to verify the absence of contamination and loss. If blanks indicate contamination, and it is determined to not be a result of contaminated reagents, it may be necessary for the polypropylene sample tubes to be rinsed and stored in dilute acid prior to use.

Note: Chromic acid must not be used for cleaning glassware for trace metals analysis.

- 4.3 Memory effects are a major concern when Hg is analyzed by conventionally nebulized ICP-MS. Memory interferences occur when elements in a previous sample contribute to signals measured in a subsequent sample. Hg can be either absorbed onto the spray chamber walls or retained as vapor in the dead volume of the spray chamber. The possibility of Hg memory interferences within an analytical run should be recognized and suitable rinse solvents and times should be used to reduce the effects. Routine maintenance (cleaning and/or replacement) of sample introduction components is necessary for long-term minimization of memory effects. Memory effects can be evaluated by using a minimum of three replicate integrations for data acquisition: A high relative standard deviation (%RSD) of the three replicates caused by successively decreasing signal intensity is indicative of carryover from the previous sample. If memory interference is suspected, the sample should be re-prepared and re-analyzed after analysis of a blank indicates that the carryover issue has been eliminated.

5.0 SAFETY

- 5.1 The toxicity or carcinogenicity of reagents and chemicals used in this method has not been fully established. Each chemical should be regarded as a potential health hazard, and exposure to these compounds should be as low as reasonably achievable.
- 5.2 Analysts who work in the lab are required to read the following MDH safety policies located in the MDH Policy and Procedure Manual:

<u>POLICY #</u>	<u>TITLE</u>
902.02	Occupational Safety and Health
420.01	Right-to-Know

In addition, the analyst should read the MDH Public Health Laboratory Division - Chemical Hygiene Plan. Questions regarding the Chemical Hygiene Plan should be referred to the Health and Safety Officer.

- 5.3 Before operating the instrument, read the information in the PerkinElmer ELAN®ICP-MS System Safety Manual. Possible hazards include ultraviolet radiation, high voltages, radio-frequency radiation, and high temperature.
- 5.4 Wear gloves, a lab coat, and safety glasses while handling all human blood. Place all disposable plastic, glass, and paper (pipette tips, auto sampler tubes, gloves, etc.) that contacts blood in a biohazard autoclave bag. Keep these bags in appropriate containers until they are sealed and autoclaved. Wipe down all work surfaces with 10% sodium hypochlorite solution when work is finished. Use the foot pedal on the Micromedic Digiflex™ to reduce analyst contact with work surfaces that have contacted blood and to free the analyst's hands to hold the specimen cups and auto sampler tubes and to wipe off the tip of the Micromedic Digiflex™.
- 5.5 Observe universal precautions. Dispose of all biological samples and diluted specimens in a biohazard autoclave bag at the end of the analysis according to CDC/DLS guidelines for disposal of hazardous waste.
- 5.6 Be especially cautious when dealing with solutions/standards containing mercury/methylmercury. Perform sample and standard preparation in a well ventilated cabinet. Mercury spill cleanup kits/reagents (powder sulfur, etc) should be available at all times.

- 5.7 Exercise special care when handling and dispensing concentrated nitric acid. Always remember to add acid to water. Nitric acid is a caustic chemical that is capable of causing severe eye and skin damage. Wear metal-free gloves, a lab coat, and safety glasses. **If nitric acid comes in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.**
- 5.8 The analyst may contact the MDH Employee Health and Safety Information Hotline regarding chemicals used in this procedure by calling the number posted in the laboratory.
- 5.9 The following guidelines are designed to aid the analyst in the safe operation of the atomic spectroscopy instrumentation and ancillary equipment:
- 5.9.1 Read and review all hazard and safety sections in the manufacturer's reference/operating manuals. Particular attention should be given to areas that are highlighted, such as those labeled: "Warning", "Important", or "Note".
- Warning:** Usually indicates an operation that could cause personal injury if precautions are not followed.
- Important:** Usually indicates an operation that could cause instrument damage if precautions are not followed.
- Note:** Usually indicates additional significant information is provided with the procedures.
- 5.9.2 Since high pressure gas cylinders are commonly used with atomic spectroscopy instrumentation, the analyst should be familiar with safe handling practices regarding the use of these cylinders.
- 5.9.3 Analytical plasma sources emit radio frequency radiation and intense UV radiation. Suitable precautions should be taken to protect the analyst from such hazards.

6.0 EQUIPMENT AND SUPPLIES

- 6.1 Calibrated mechanical pipettes in the following ranges:
- 10-100 µL
100-1000 µL

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1000-5000 µL

- 6.2 Trace metal grade pipette tips.
- 6.3 Talc-free gloves.
- 6.4 Falcon 15-mL conical tubes (#2097) and 50-mL conical tubes (#2098) (Becton, Dickinson Labware, Franklin Lakes, NJ). The tubes should be lot screened for blank mercury content before use.
- 6.5 Falcon Pro-Bind™ 96 Well Assay Plates (#3910), U-bottom without lid, polystyrene, non-sterile.
- 6.6 Millex MultiScreen™-BV 96 Well filter plates (cat. # MABVN1210), 1.2 µm Hydrophilic, low protein binding, Durapore® Membrane.
- 6.7 BioExpress® X-Pierce™ pre-cut pierceable films for 96 well plates, non-sterile (XP-100).
- 6.8 Wallac Delfia® Plateshake (#1296-002), 96 well plate agitator.
- 6.9 Pall Corporation Multi-well plate vacuum manifold (#5017).
- 6.10 Teflon™ or Nalgene Polypropylene (PP) 1-L and 2-L containers.
- 6.11 Volumetric flasks, 100mL (glass).
- 6.12 Argon gas supply (high purity grade gas or liquid, 99.99%).
- 6.13 Kaydry™ paper towels and Kimwipe™ tissues (Kimberly-Clark Corp., Roswell, GA).
- 6.14 ICP-MS system
 - 6.14.1 Inductively Coupled Plasma-Mass Spectrometer ELAN® ICPMS DRC II (PerkinElmer, Norwalk, CT) equipped with
 - 6.14.1.1 ESI SC-FAST autosampler with ESI SC series Autosampler probe (ES-5037-3250-080), 0.25 mm i.d. × 80 cm long, flanged for ST type nebulizers and green/orange peristaltic pump tubing (PerkinElmer N0777042)
 - 6.14.1.2 Quartz Cyclonic Spray Chamber (part number N0777035)

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-
- 6.14.1.3 PFA/Quartz MicroFlow Nebulizer (part number N8122192)
- 6.14.1.4 PFA/Quartz Torch Injector Assembly (part number N8122394).
- 6.14.1.5 This is the preferred sample introduction system for this method, due to its lower “noise” level and superior limits of detection. Parameters of x-y alignment, mass calibration, auto lens voltages, and nebulizer gas flow rates are optimized regularly as described in the ELAN® ICPMS Inductively Coupled Plasma-Mass Spectrometer Software Guide. The instrument contains a radio-frequency generator which is compliant with FCC regulations. Solution delivery to the nebulizer is accomplished by using a variable-speed peristaltic pump. Constant flow of the nebulizer gas supply is maintained by a mass-flow controller. A preferred ICP-MS operating setting is outlined in Table 1.

Table 1. Multi-element ICP-MS ELAN® ICPMS Settings

Parameter	Setting
RF power	1.45 KW
Ar nebulizer gas flow	0.90-1.15 LPM
Detector mode	Pulse
Measurement units	Cps
Replicates	3
Readings/replicate	1
Auto lens	On
Blank subtraction	After internal standard
Curve type	Simple Linear
Sample units	ng/L
Sweeps/reading	20
Dwell time	Ir 40ms Hg 75ms
Integration time	Ir 800 ms Hg 1500 ms

6.14.2 Instrument Consumables (see manufacturer’s literature).

7.0 REAGENTS AND STANDARDS

- 7.1 Reagent Water: ASTM Type I (ASTM D 1193) or equivalent with a resistivity > 18 MΩ-cm at 25° C and free of the analytes of interest. All the water used in this SOP is reagent water.
- 7.2 Only “Analytical Reagent Grade” (AR) or American Chemical Society (ACS) grade chemicals should be used. All the reagents should be free of Hg.
- 7.3 Reagents may contain impurities, which can affect the integrity of the analytical results. Due to the high sensitivity of ICP-MS, high-purity reagents must be used whenever possible. All acids must be ultra high purity grade. Nitric acid is preferred for ICP-MS in order to minimize polyatomic interferences.
- 7.4 Nitric Acid (“AR Select™ Plus,” Mallinckrodt-Baker, 500 mL in poly coated glass).
- 7.5 Hydrochloric Acid: analytical grade or above
- 7.6 Triton X-100™ (“Baker Analyzed,” J.T. Baker Chemical Co.).
- 7.7 L-Cysteine (99%, Alfa Aesar, Catalog #: A10435).
- 7.8 2-Mercaptoethanol (98%, Alfa Aesar, Catalog#: A15890).
- 7.9 Methanol: analytical grade or above
- 7.10 EDTA: analytical grade or above
- 7.11 Tuning and Daily Performance Solution: 1 µg/L Mg, Co, Fe, In, Ce, Pb, U and Th and 10 µg/L Ba and Be in 1% HNO₃. This solution is used to verify instrument tune and mass calibration prior to analysis.
- 7.12 Internal Standard Stock Solutions: Ir: SPEX PLIR3-2Y, 1,000 mg/L (SPEX Industries, Inc., Edison, NJ), or equivalent.
- 7.13 Standard Hg Stock Solution: Hg 10 µg/mL (in 10% HCl) (Inorganic Ventures, MSHG-10PPM), or equivalent.
- 7.14 Standard Hg Stock Solution: Hg 1000 mg/L (as Hg²⁺ in 5% HNO₃) (SpecPure^R), or equivalent.

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- 7.15 Standard Methylmercury Stock Solution: Methylmercury (as CH₃HgCl in water), containing Hg 1000 mg/L (Alfa Aesar), two different lot numbers, or equivalent.
 - 7.16 NIST Standard Reference Material 966: Toxic Metals in Bovine Blood Level 2 (National Institute of Standards and Technology, Gaithersburg, MD), containing 31.4 ± 1.7 µg/L Hg.
 - 7.17 Base Blood: The base blood used in this method is a pool of blood purchased from a commercial blood bank and preserved with EDTA. Collect blood in lot screened or acid-rinsed sample collection cups. For long-term storage, store at ≤ 20°C.
 - 7.18 Aqueous Blank: The aqueous blank (AB) contains all aqueous reagents in reagent water and is used to establish the analytical calibration curve and to assess possible contamination from the aqueous reagents prepared for this method.
 - 7.19 Method Blank: The laboratory method blank (MB) is blank filter paper punches from each sample and is used as a blank for the sample preparation process and to determine any background Hg in the filter paper.

8.0 SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

- 8.1 Please refer to the separate MDH SOP, "Method of Punching Dried Blood Spots on Filter Paper for Mercury Biomonitoring in Newborns in the Lake Superior Basin and other Minnesota Locations," for information on sample collection, preservation and storage.
- 8.2 Once the sample punches are received at the Minnesota Laboratory they will be logged into the Laboratory LIMS system. Each specimen ID will receive a unique LIMS number and then be transferred to the Inorganic Chemistry Unit. An internal chain of custody procedure documenting the location of the sample punches will be maintained. The sample punches will be stored in a locked freezer located in the sample chain of custody room L250.

9.0 PREPARATION OF REAGENTS, CALIBRATION STANDARDS, CONTROLS

- 9.1 Reagent Preparation
 - 9.1.1 20 mg/L Ir: The intermediate internal standard stock solution is an aqueous solution containing 20 mg/L Ir in 2% (v/v) double-distilled nitric acid. To a 50-mL container that is partially filled with reagent water, add

1 mL of the internal standard stock solution (Section 7.12) and 1 mL double-distilled nitric acid (Section 7.4). Dilute to volume with reagent water.

- 9.1.2 2% Hydrochloric Acid Solution: Prepare by partially filling a 1-L container with reagent water and adding 20 mL hydrochloric acid (Section 7.5). Dilute to volume with reagent water.
- 9.1.3 Diluent: The extracting solvent/diluent used in this method is an aqueous solution containing 10µg/L Ir, 0.05% (v/v) 2-mercaptoethanol, 0.005% (w/v) L-cysteine, 0.005% (w/v) EDTA, and 0.01% (v/v) Triton X-100. Add this solution when preparing samples during the dilution process, just prior to analysis. To prepare, acid-rinse a 2-L container (polypropylene (PP), polymethylpentene (PMP), or Teflon™) and partially fill it with reagent water. Add 1 mL of 20 mg/L Ir (Section 9.1.1), 1 mL 2-mercaptoethanol (Section 7.8), 100 mg L-cysteine (Section 7.7), 100 mg EDTA (Section 7.10), and 100µL Triton X-100 (Section 7.6). Dilute to volume with reagent water.
- 9.1.4 ICP-MS Rinse Solution: The rinse solution used in this method is especially designed to reduce or eliminate the significant memory effect associated with Hg analysis by nebulizer sample introduction. It is an aqueous solution containing 4% methanol (Section 7.9), 0.002% Triton X-100™ (Section 7.6) and 1% (v/v) hydrochloric acid (Section 7.5). Pump this solution into the sample introduction system between samples to prevent carry-over of mercury from one sample measurement to the next. To facilitate the day-to-day preparation of the rinse solution, first prepare a 2% Triton X-100™/1% (v/v) nitric acid solution by adding 40 mL of Triton X-100™ (Section 7.6) and 20 mL of nitric acid (Section 7.4) to a 2-L, acid-washed bottle (PP, PMP, or Teflon™) that is partially filled with reagent water, fill to 2 L, and stir or shake well until the Triton X-100™ has completely dissolved in the solution. Prepare the final rinse solution by acid-rinsing a 2-L container (PP, PMP, or Teflon™) and partially filling it with reagent water. Add 20 mL of hydrochloric acid (Section 7.5), 80 mL methanol (Section 7.9), and 2.0 mL of the 2% Triton X-100™ / 1% (v/v) nitric-acid solution. Dilute to 1 L using reagent water. Store at room temperature and prepare as needed. The rinse solution is useable for 6 months after preparation.

9.2 Standards Preparation

- 9.2.1 Calibration standards must always be traceable to the National Institute for Standards and Technology (NIST). Anytime new calibration standards are used, verify that the calibration information in the ELAN® software (method window, calibration page) reflects the actual calibrator concentrations.
- 9.2.2 Aqueous Mercury Intermediate Working Calibration Standard Stock: The intermediate working standard stock solution is an aqueous solution prepared from stock solutions of mercury (Sections 7.13 and 7.15) and is used as the stock solution for the serial dilutions for preparing the intermediate working calibration standards. The solution matrix is 2% nitric acid and 1% hydrochloric acid in reagent water. To prepare: acid-rinse one 100-mL volumetric flask and partially fill with reagent water. To the flask add 2 mL of concentrated nitric acid (Section 7.4) and 1 mL of concentrated hydrochloric acid (Section 7.5). Add 2.5 mL of the inorganic mercury stock standard (Section 7.13) and 25 µL of the methylmercury stock standard solution (Section 7.15) and bring to volume with reagent water. Transfer to an acid-cleaned, labeled, Teflon™ container for storage. Store the solution at room temperature. The concentration of total mercury in solution is 500 µg/L.
- 9.2.3 Aqueous Mercury Intermediate Working Calibration Check Standard Stock: The intermediate working calibration check standard stock solution is an aqueous solution prepared from stock standard solutions of mercury (Sections 7.14 and 7.15) and is used as the stock solution for the dilution to prepare the intermediate working calibration check standard. The solution matrix is 2% nitric acid and 1% hydrochloric acid in reagent water. To prepare: acid-rinse one 100-mL volumetric flask and partially fill with reagent water. To the flask add 2 mL of concentrated nitric acid (Section 7.4) and 1 mL of concentrated hydrochloric acid (Section 7.5). Add 25 µL of each mercury stock standard solution (Sections 7.14 and 7.15) and bring to volume with reagent water. Be sure to use a different lot number of the methylmercury standard (Section 7.15) than was used for the aqueous mercury intermediate working calibration standard stock (Section 9.2.2). Transfer to an acid-cleaned, labeled, Teflon™ container for storage. Store the solution at room temperature. The concentration of total mercury in solution is 500 µL.

9.2.4 Aqueous Mercury Intermediate Working Calibration Standards: The intermediate working standard solutions used in this method are aqueous solutions prepared from serial dilutions of the aqueous mercury intermediate working calibration standard stock (Section 9.2.2). The solution matrix is 2% nitric acid and 1% HCl in reagent water. To prepare: acid-rinse one 100-mL glass volumetric flask for each working standard solution and partially fill with reagent water. To each 100-mL flask add 2 mL of concentrated nitric acid (Section 7.4) and 1 mL of concentrated hydrochloric acid (Section 7.5). Add the appropriate aliquot of the aqueous mercury intermediate working calibration standard stock (Table 2) and bring to volume with reagent water. Transfer to acid-cleaned, labeled, Teflon[™] containers for storage. Store the solutions at room temperature. Use these solutions each day of analysis to prepare the final working aqueous calibration standards (Section 11.2.3) that will be placed in the appropriate wells of the 96-well plate to be analyzed by the ELAN® ICPMS. See Table 2 for standard preparation instructions.

Table 2. Aqueous Mercury Intermediate Working Calibration Standards preparation

	Volume of Aqueous Mercury intermediate stock	Final Conc. (µg/L total Hg)
Inter. STD5	20 mL	100
Inter. STD4	10 mL	50
Inter. STD3	2 mL	10
Inter. STD2	1.5 mL	7.5
Inter. STD1	1 mL	5

9.2.5 Aqueous Mercury Intermediate Working Calibration Check Standard: The intermediate working calibration check standard solution used in this method is an aqueous solution prepared from a dilution of the aqueous mercury intermediate working calibration check standard stock (Section 9.2.3). The solution matrix is 2% nitric acid and 1% hydrochloric acid in reagent water. To prepare: acid-rinse one 100-mL glass volumetric flask and partially fill with reagent water. To the flask add 2 mL of concentrated nitric acid (Section 7.4) and 1 mL of concentrated hydrochloric acid (Section 7.5). Add 2 mL of the aqueous mercury intermediate working calibration check standard stock and bring to volume with reagent water. Transfer to an acid-cleaned, labeled, Teflon[™] container for storage at room temperature. Use this solution each day of analysis to prepare the final working aqueous calibration check standard

(Section 11.2.3) that will be placed in the appropriate well of the 96-well plate to be analyzed by the ELAN® ICPMS.

- 9.2.6 Working Aqueous Calibration Standards: The working aqueous calibration standard solutions used in this method are a series of dilutions from the Aqueous Mercury Intermediate Working Calibration Standards (see Section 9.2.4). These are prepared at the time of analysis (see Section 11.2.3) by adding aliquots of the aqueous mercury intermediate working calibration standards (Sec. 9.2.4) to diluent.
- 9.2.7 Working Aqueous Calibration Check Standard; the working aqueous calibration check standard used in this method is a dilution from the Aqueous Mercury Intermediate Working Calibration Check Standard (Section 9.2.5). It is prepared at the time of analysis (see Section 11.2.3) by adding aliquots of the aqueous mercury intermediate working calibration check standard (Section 9.2.5) to diluent (Section 9.1.3).
- 9.2.8 Aqueous-Based Calibration Verification Standards (CVS)
- 9.2.8.1 Calibration verification materials can be either purchased from an external laboratory or prepared within the MDH laboratories. Calibration verification must always be traceable to the National Institute for Standards and Technology (NIST). The MDH laboratory currently prepares its own calibration verification standards.
- 9.2.8.2 Aqueous Intermediate Working Low, Medium, and High CV Standards (aqueous based): The aqueous intermediate working CV solutions used in this method are three aqueous solutions of mercury in 2% (v/v) nitric acid and 1% (v/v) hydrochloric acid, with concentrations different from the calibration standards. Use these solutions on each day of analysis to prepare the final working CVS that will be placed in the auto sampler of the ELAN® ICPMS. Prepare the CVS by acid-rinsing three 100-mL volumetric flasks and partially fill them with reagent water, add 2 mL of concentrated nitric acid (Section 7.4) and 1 mL of concentrated hydrochloric acid (Section 7.5). Add the appropriate aliquot of the aqueous mercury intermediate working calibration standard stock (Section 9.2.2) (Table 3) and bring to volume with reagent water. Transfer to 100-mL, acid-cleaned PP containers for

storage. The final concentration of total Hg in each solution is listed in Table 3.

Table 3: Concentration of Aqueous Intermediate CV Standards

	Volume of Aqueous Mercury intermediate stock	Concentration (µg/L total Hg)
Inter. high CVS	15.00	75
Inter. medium CVS	4.00	20
Inter. low CVS	1.20	6

9.2.8.3 Working CV Low, Medium, and High Standards (aqueous based): The working aqueous mercury CVS used in this method are appropriate dilutions of the aqueous intermediate working low, medium, and high CVS (Section 9.2.8.2). The aqueous intermediate CVS are diluted with diluent at the time of analysis (see section 11.2.3).

9.2.9 Blood-Based Quality Control Sample (QCS)

9.2.9.1 The SRM 966 Level 2 from the National Institute for Standards and Technology (Section 7.16) is bovine blood with a certified value of total mercury and is used as the QCS for this method.

9.2.9.2 Blood-Based QC Sample Preparation: The Blood-Based QCS (SRM 966 Level 2, Section 7.16) are diluted to a final concentration of 0.659 µg/L with diluent (Section 9.1.3) and 2% hydrochloric acid (Section 9.1.2) at the time of analysis (see Section 11.2.4). This dilution is used to be consistent with dried bloodspot samples.

9.2.10 Laboratory Control Sample (LCS)

9.2.10.1 The Laboratory Control Sample (LCS) will use the SRM 966 Level 2 from the National Institute for Standards and Technology (Section 7.16) as a certified mercury source of known concentration to assess recovery through the extraction process.

9.2.10.2 LCS Preparation: Personnel in the Environmental Laboratory at the Minnesota Department of Health apply spots of SRM 966 Level 2 (Section 7.16) to blank filter paper cards. The blood spots are allowed to air-dry for several hours after which 3 mm blood spots are punched from the cards according to the separate SOP, "Method of Punching Dried Blood Spots on Filter Paper for Mercury Biomonitoring in Newborns in the Lake Superior Basin and Other Minnesota Locations,".

9.2.10.3 An LCS is carried through the extraction and sample preparation procedure as outlined in Section 11.2.6.

9.2.11 Aqueous Blank Sample

9.2.11.1 The Aqueous Blank (AB) sample will be used as a calibration blank to establish the zero point for the analytical calibration curve. It also serves as the blank for the aqueous-based CV standards and to account for any mercury contamination of the labware or reagents used. It is subtracted from all patient samples.

9.2.11.2 The AB sample is prepared at the time of analysis as described in Section 11.2.2. It consists of reagent water (Section 7.1), 2% hydrochloric acid (Section 9.1.2), and diluent (Section 9.1.3).

9.2.12 Method Blank

9.2.12.1 The Method Blank (MB) is used as a blank for the sample preparation process and to determine any background Hg in the filter paper.

9.2.12.2 MB samples will be prepared along side blood samples by punching blank spots from the filter paper cards according to the separate SOP, "Method of Punching Dried Blood Spots on Filter Paper for Mercury Biomonitoring in Newborns in the Lake Superior Basin and Other Minnesota Locations." These blank samples will be carried through the extraction and sample preparation procedure as outlined in Section 11.2.5.

10.0 CALIBRATION AND CALIBRATION VERIFICATION PROCEDURE

10.1 Calibration Curve

- 10.1.1 Generate a simple linear calibration curve for total mercury using external standards prepared in aqueous diluent with concentrations as defined in Section 11.2.3.
- 10.1.2 The calibration used for this analysis must be simple linear and not forced through zero. The calibration standard curve is accepted if the correlation coefficient of 0.999 is achieved.
- 10.1.3 The ratio of analyte and internal standard intensities is used to determine the net intensity for the analyte. The internal standard allows for the correction of changes in instrument hardware response or for sample-to-sample variations in sensitivity.
- 10.1.4 Aqueous blank subtraction is performed after the analyte / internal standard ratio is calculated.

10.2 Calibration Verification

- 10.2.1 In order to verify that the calibration of the test system is accurate throughout the reportable range, high, medium, and low calibration verification (CV) standards in aqueous diluent (Section 11.2.3) will be analyzed immediately following the calibration standards, after every 20 samples, and at the end of the analytical run.
- 10.2.2 In addition, a calibration check standard, which is made using a different stock solution from the calibration curve, will be analyzed immediately following the calibration standards.

11.0 OPERATING PROCEDURE

11.1 Preliminaries

- 11.1.1 For information regarding the reportable range of results and how to handle results outside this range, refer to “Reportable Range of Results” (See Section 11.5.).
- 11.1.2 Allow frozen and refrigerated blood spot specimens and QC spot specimens to reach ambient temperature.

11.2 Sample Preparation

- 11.2.1 Set up a series of 15-mL polypropylene tubes corresponding to the number of blanks, calibration standards, calibration check standard, CVS, and QCS.
- 11.2.2 Prepare an aqueous blank (AB) consisting of 40 μ L of water, 1960 μ L of 2% hydrochloric acid (Section 9.1.2) and 2000 μ L of diluent (Section 9.1.3). This will be used as the blank for the calibration curve and as the blank for reagents and labware. It will also be analyzed after standard 5 and after every 20 samples as an aqueous blank. Volumes may be increased proportionally for larger sample batches.
- 11.2.3 Prepare the working calibration standards, calibration check standard, and aqueous-based CV standards consisting of 20 μ L of the appropriate aqueous intermediate working calibration standard (Section 9.2.4), aqueous intermediate working calibration check standard (Section 9.2.5), or aqueous intermediate CV standard (Section 9.2.8.2), 980 μ L of 2% HCl (Section 9.1.2) and 1000 μ L of diluent (Section 9.1.3). Volumes may be increased proportionally for larger sample batches. The final concentration of working calibration standards and CVS are listed in table 4:

Table 4: Concentrations of working calibration standards

Standards	Concentration (μ g/L total Hg)
Working STD1	0.05
Working STD2	0.075
Working STD3	0.1
Working STD4	0.5
Working STD5	1
Check Standard	0.1
High CVS	0.75
Medium CVS	0.2
Low CVS	0.06

- 11.2.4 Prepare the Blood-based QCS using 21 μ L SRM 966 level 2 (Section 7.16), 500 μ L diluent (Section 9.1.3), and 479 μ L 2% HCl (Section 9.1.2).

Volumes may be increased proportionally for larger sample batches. Final total mercury concentration in the QCS is listed in Table 5:

Table 5: Concentrations of QCS.

QCS	Concentration (µg/L total Hg)
SRM 966 Level 2	0.659

- 11.2.5 Prepare the method blank (MB) sample for each patient sample as 150 µL of diluent (Section 9.1.3), two 3mm blank filter paper spots, and 150 µL of 2 % HCl (Section 9.1.2) in a well in the 96-well filter plate.
- 11.2.6 Prepare the laboratory control sample (LCS) using 150 µL of diluent (Section 9.1.3), two 3mm blood spots containing SRM 966 Level 2 as the blood source (9.2.10.2), and 150 µL of 2 % HCl (Section 9.1.2).
- 11.2.7 Prepare the patient blood sample dilutions as 150 µL of diluent (Section 9.1.3), two 3mm blood spots, and 150µL of 2% HCl (Section 9.1.2).
- 11.2.8 Add 300 µL of each calibration curve standard, blank, calibration check standard, QCS, and CVS to designated wells in the 96-well filter plate along with two 3mm blank filter paper spots. Cover filter plate with lid and agitate on plate agitator for approximately 30 minutes on slow. Allow plate to sit at room temperature overnight setting the filter plate over a lid containing a thin layer of reagent water and being careful not to allow the filter tips to touch the water's surface. The humid environment created by the reagent water acts to eliminate clogs in the filter tips of the filter plate that may form and dry overnight due to capillary action in the filter apparatus..
- 11.2.9 The next day agitate the plate for an addition 20 minutes and then filter into a U-bottom 96-well plate using the Multi-well plate vacuum manifold. Cover the 96-well plate with a piece of BioExpress® X-Pierce™ pre-cut pierceable film. Place the 96-well plate in the autosampler of the ELAN® ICPMS.
- 11.3 Instrument and Software Setup for the ICP-MS
- 11.3.1 Turn on the computer, printer, peristaltic pump, and auto sampler, and log into the operating system.

- 11.3.2 Set up the peristaltic pump tubing for the sample rinse station, positioning the tubing and closing the pump clamps.
- 11.3.3 Start the ELAN[®] ICPMS and ESI autosampler software from Windows[™].
- 11.3.4 Perform necessary daily maintenance checks as described in the *ELAN[®] ICPMS Hardware Guide* (i.e., Argon supply pressure and tank level, cleanliness and positioning of interface components, interface pump oil condition, etc.). Note the base vacuum pressure in the INSTRUMENT window of the software (before igniting the plasma, the vacuum is typically between 8×10^{-7} and 1.8×10^{-6} torr). Record any maintenance procedures, along with the base vacuum pressure, in the *Daily Maintenance Checklist Logbook*.
- 11.3.5 In the INSTRUMENT window of the software, press the “Start” button to ignite the ELAN[®] ICPMS plasma.
- 11.3.6 Start the peristaltic pump by pressing the appropriate arrow in the DEVICES window (make sure that the rotational direction is correct for the way the tubing is set up in the peristaltic pump). Set the pump speed to 12 rpm in the DEVICES window.
- 11.3.7 Allow at least 45 minutes warm-up time for the ICP-MS. After this warm-up time, complete the appropriate daily optimization procedures as described in the *ELAN[®] ICPMS Inductively Coupled Plasma-Mass Spectrometer Software Guide*, including mass calibration and resolution, lens voltage, autolens, nebulizer gas optimization, and daily performance. You may wish to include Be (m/z 9) in the daily performance check, although performance specifications for this element are not available from the manufacturer. If the performance is satisfactory (see Table 6), proceed to the analysis of samples. If not, use the Smart Tune Wizard to re-optimize the instrument, followed by an additional daily performance check. Fill in the *Daily Maintenance Checklist Logbook* according to the optimization procedures performed. Save new tuning (mass calibration) parameters to the file “default.tun.” Save new optimization parameters (i.e., detector voltages, auto lens values, nebulizer gas flow rate) to the file “default be 12.dac.” Monthly or any time large changes are made in optimization parameters, save a separate copy of these optimization files under a different name (i.e. – default_070703.dac). Suggested tune Specifications are listed in table 6:

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Table 6: Daily performance criteria

Sensitivity	Mg	>7000 cps/1ppb
	In	>30000cps/1ppb
	U	>30000cps/1ppb
Precision (relative standard deviation of replicates)		< 5%
Oxides (CeO / Ce)		≤ 4.0
Background (at 220amu)		<2.0 cps
Mass calibration/accuracy		±0.05amu
Mass Resolution		0.65-0.75 amu at 10% peak height

11.3.8 To set up the run in the software, click on “Open Workspace” from the “File” menu. Select the workspace file “Hg in bloodspots.wrk”. Select “Review Files” from the “File” menu. From this window, you will be able to set up the correct files and data directories for your analysis.

Method: hg in filter paper_96 well_low curve.mth.

Dataset: The data set should be in C:\elandata\Dataset\Hg in Bloodspots\ and backed up in ‘PHL Shared Drive’ S:\Metals\DRC II data

Sample: If an analysis has been performed that is similar to the one being set up, select the sample file corresponding to it and edit it for the present analysis.

Report Template: Select “Hg Bloodspots.” (Note: The setting in the method file used during analysis will supersede your selection here.)

Tuning: “default be 12.tun” (Note: The setting in the method file used during analysis will supersede your selection here.)

Optimization: “default.dac” (Note: The setting in the method file used during analysis will supersede your selection here.)

Calibration: No file needed.

Polyatomic: elan.ply

11.3.9 In the ESI software, check that the FAST Control Enabled box is unchecked. The Rinse settings can be found in Table 7 below. The “Max Rinse Time” Enabled box should be checked with the time listed as 300 sec.

Table 7. ESI Autosampler Rinse Settings.

	Rinse Time (sec)	Additional Flush Time (sec)
Rinse 1	130	0
Rinse 2	0	0

11.3.10 In the SAMPLES/BATCH window, update the window to reflect the current sample set (i.e., auto sampler locations, sample identification (id), analysis methods, peristaltic pump speeds, etc.). A typical SAMPLE/BATCH window for this method will look like the following:

Table 8: A sample of SAMPLE/BATCH window setup

A/S* Location	Sample ID	Measurement Action	Method
301	BlankChk	Run blank, standards and sample	hg in filter paper_96 well_low curve.mth
307	Blank	Run sample	hg in filter paper_96 well_low curve.mth
308	Check Std	Run sample	hg in filter paper_96 well_low curve.mth
309	Blank	Run sample	hg in filter paper_96 well_low curve.mth
310	Low CVS	Run sample	hg in filter paper_96 well_low curve.mth
311	Med. CVS	Run sample	hg in filter paper_96 well_low curve.mth
312	High CVS	Run sample	hg in filter paper_96 well_low curve.mth
313	Blank	Run sample	hg in filter paper_96 well_low curve.mth
314	Blank	Run sample	hg in filter paper_96 well_low curve.mth
315	RLV	Run sample	hg in filter paper_96 well_low curve.mth
316	QCS	Run sample	hg in filter paper_96 well_low curve.mth
317	LCS	Run sample	hg in filter paper_96 well_low curve.mth

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318	LCS Blank	Run sample	hg in filter paper_96 well_low curve.mth
319	LCS Blank	Run sample	hg in filter paper_96 well_low curve.mth
320	PT 0948	Run sample	hg in filter paper_96 well_low curve.mth
321	PT 0948 Blank	Run sample	hg in filter paper_96 well_low curve.mth
322	PT 0948 Dup	Run sample	hg in filter paper_96 well_low curve.mth
323	PT 0948 Dup Blank	Run sample	hg in filter paper_96 well_low curve.mth
324-343	Samples and Blanks	Run sample	hg in filter paper_96 well_low curve.mth
344	CVS Low	Run sample	hg in filter paper_96 well_low curve.mth
345	CVS Med	Run sample	hg in filter paper_96 well_low curve.mth
346	CVS High	Run sample	hg in filter paper_96 well_low curve.mth
347	Blank	Run sample	hg in filter paper_96 well_low curve.mth
348	Blank	Run sample	hg in filter paper_96 well_low curve.mth
349	QCS	Run sample	hg in filter paper_96 well_low curve.mth
350	LCS	Run sample	hg in filter paper_96 well_low curve.mth
351	LCS Blank	Run sample	hg in filter paper_96 well_low curve.mth
352	LCS Blank	Run sample	hg in filter paper_96 well_low curve.mth
353-372	Samples and Blanks	Run sample	hg in filter paper_96 well_low curve.mth
373	Field Dup Sample	Run sample	hg in filter paper_96 well_low curve.mth
374	Field Dup Blank	Run sample	hg in filter paper_96 well_low curve.mth
375	QCS	Run sample	hg in filter paper_96 well_low curve.mth
376	LCS	Run sample	hg in filter paper_96 well_low curve.mth
377	LCS Blank	Run sample	hg in filter paper_96 well_low curve.mth
378	LCS Blank	Run sample	hg in filter paper_96 well_low curve.mth
379	PT 0949	Run sample	hg in filter paper_96 well_low curve.mth

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380	PT 0949 Blank	Run sample	hg in filter paper_96 well_low curve.mth
381	PT 0949 Dup	Run sample	hg in filter paper_96 well_low curve.mth
382	PT 0949 Dup Blank	Run sample	hg in filter paper_96 well_low curve.mth
383	CVS Low	Run sample	hg in filter paper_96 well_low curve.mth
384	CVS Med	Run sample	hg in filter paper_96 well_low curve.mth
385	CVS High	Run sample	hg in filter paper_96 well_low curve.mth
386	Blank	Run sample	hg in filter paper_96 well_low curve.mth
387	Blank	Run sample	hg in filter paper_96 well_low curve.mth

* The auto sampler positions of CVS, QCS, and blood samples do not have to be those shown above, but the order in which these are run should be as shown above.

Use the following settings for uptake and rinse times for all samples. (These values are already stored in the method files for the blanks and standards.)

Table 9. Settings for Uptake and Rinse Times

	Pump Speed	Duration
Sample flush	-12 rpm	65 seconds
Read delay and analysis	-12 rpm	20 seconds
Wash	-12 rpm	130 seconds

The ELAN[®] software can be used to automatically correct for sample dilutions. If this function is desired, specify the dilution factor in the SAMPLE/BATCH window.

11.3.11 Once you have edited the parameters in the SAMPLE/BATCH window for your run, place solutions in the auto sampler tray according to the setup of the SAMPLE/BATCH window and method files. Highlight (click and drag with the mouse) the table rows of the samples that should be included in the run, and then click on “Analyze Batch.” The Scheduler may also be used to start a run. Click on SCHEDULER and check the

boxes for Analyze Samples, Wash, and AutoStop. Specify the Sample and Dataset files and click “Start” to start the run. The instrument will shut down automatically when the run is complete.

11.4 Calculations

11.4.1 The ELAN[®] has two onboard microcomputers that work with the external system computer. The computers interface with the other electronic components within the system to convert the detector signals to digital-ion-intensity values. An internal standard (Ir) is used in the analysis, which allows for the correction of changes in instrument response and sample matrix. The software uses the ratio of analyte and internal standard intensities to determine the net intensity for the analyte. As standard solutions are analyzed, the software plots the net intensity versus the concentration for mercury in the standard solution. The calibration curve is updated as each subsequent standard is analyzed. The resulting calibration curve is used as a reference to determine the concentration of mercury in each sample based on the aqueous blank corrected ratio of the intensities of mercury and the internal standard observed in the samples.

11.4.2 The method detection limit (MDL) for total mercury in dried bloodspots is determined by multiplying the standard deviation of 10 analyses of spiked base blood at a level equivalent to the low calibration standard (2.42 µg/L Hg) that has been applied to filter paper cards with the Student’s t value. The MDL was determined to be 0.791 µg/L total Hg in blood. The report level is set to 2.42 µg/L total Hg in blood. Results for samples in which mercury is not detected above the MDL are reported as “< 2.42 ug/L.” Results for samples in which mercury is detected between the MDL and Report Level will be reported as positive values, however with a “J” flag qualifier signifying that the result is an estimated value.

11.4.3 Concentration of total mercury in blood spots: By taking into account the final volume of solution, any subsequent dilutions, and the volume of blood on each punch, the concentration of total mercury for each sample may be calculated from the mercury concentration determined by the calibration curve less the aqueous blank (AB) using the following equation:

$$FC = \frac{C_s \times D \times V_f}{V_B}$$

Where:

FC = final concentration of total mercury

Cs = concentration of total mercury determined from calibration curve less the AB

D = dilution factor, (will almost always equal 1)

V_f = final volume, equal to 300 µL

V_B = initial volume of blood, equal to 6.2 µL, as each blood spot contains 3.1 µL blood

11.4.4 Recovery: Percent recovery for the LCS may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{C_s - C}{S} \times 100$$

where:

R = percent recovery

C_s = fortified sample concentration

C = sample background concentration

S = concentration equivalent of fortifier added to sample

11.4.5 Accuracy: The accuracy of the LCS as percent recovery is calculated using the following formula:

$$\text{Accuracy (\%)} = (X_{\text{Found}}/TV) \times 100$$

11.4.5.1 If the LCS recovery of mercury falls outside the control limits of 80-120%, that analyte is judged out of control, and the source of the problem should be identified and resolved before preparing further samples for analysis.

11.4.5.2 When sufficient internal performance data become available, develop control limits from the percent mean recovery (X) and the standard deviation (S) of the mean recovery. These data are used to establish upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = X + 3S$$

$$\text{LOWER CONTROL LIMIT} = X - 3S$$

11.4.5.3 After each five to ten new recovery measurements, new control limits should be calculated using only the most recent 20 to 30 data points. These limits must not be greater than the 80-120 %.

11.4.6 Precision: Analyze a LCS/LCSD with each set of samples processed as a group. The absolute difference between duplicates and relative percent difference (RPD) of the duplicates are calculated and used to monitor the precision of the method. The RPD should not exceed 20 %. If the difference or RPD for a set of duplicates falls outside of the applicable control limits, the reason for the out of control condition is investigated and the duplicate analyses are re-prepared and reanalyzed. The RPD may be calculated using the following equation:

$$RPD = \frac{|(C - C_{Dup})|}{((C + C_{Dup})/2)} \times 100$$

where:

RPD = Relative Percent Difference

C = sample concentration

C_{Dup} = sample duplicate concentration

11.5 Reportable Range of Results

11.5.1 Total blood mercury values are reportable in the range between the RL and the highest calibration standard (2.42 – 48.4 µg/L Hg in blood). If an analyte concentration is observed that is lower than the RL but higher than the MDL (0.791 µg/L Hg in blood), then the result is reported with a “J” flag to indicate that the value is estimated. If an analyte concentration is observed that is higher than the highest standard concentration, but below the known range of linearity of 242 µg/L, then the result will be reported as “estimated.”

11.6 Quality Control (QC) Procedures

11.6.1 The method described in this protocol is intended to be used for environmental and occupational health screening studies.

11.6.2 This analytical method uses three levels of QC sample determinations:

- 11.6.2.1 The calibration verification standards (CVS) used in this method comprise three levels of concentration spanning the “low-normal”, normal, and “high-normal” ranges of total mercury. The three CVS are analyzed after the calibration standards and before any patient-derived blood samples are analyzed. The three CVS are analyzed after every 20 samples and again at the end of the run.
- 11.6.2.2 The NIST SRM 966 Level 2 bovine blood serves as the QCS. The certified bovine blood is analyzed after CVS and before any patient-derived blood samples, after every 20 samples and again at the end of the run.
- 11.6.2.3 The LCS/LCSD uses SRM 966 Level 2 as the blood source on the filter paper cards as a measure of the complete laboratory procedure. It is analyzed at the beginning of a run, after every 20 samples and again at the end of the run.

11.7 Calibration Verification and Sample Results Evaluation

11.7.1 After completing a run, the run will be judged to be in or out of control. The CV limits are based on the average and standard deviation of the beginning and ending analyses of each of the CV pools, so it will not be possible to know if the run is officially accepted or rejected until it is completed. The CV rules applied to the data are as follows:

- 11.7.1.1 If all CV run means are within 2S limits then accept the run.
- 11.7.1.2 If one of two of the CV run means is outside a 2S limits, then apply the rules below and reject the run if any condition is met.
 - i. 1_{3S} – Average of both CV low or average of both medium or average of both high CV are outside of a 3_S limit
 - ii. 2_{2S} - Average of both CV low and average of both medium and average of both high CV are outside of 2s limits on the same side of the mean.
 - iii. R_{4S} – sequential – Average of both CV low and average of both medium and average of both high CV are outside of 2_S limit on opposite sides of the mean.

iv. 10 X-sequential – Current and previous 9 run means are on same side of the characterization mean for either the low, medium, or high.

11.7.2 Sample Results Precision Evaluation: If relative standard deviation of the three replicate readings for a single sample analysis is greater than 10%, then repeat the analysis of that sample.

11.8 Remedial Action If Calibration, CVS, QCS, or LCS/LCSD Fail to Meet Acceptable Criteria

11.8.1 Since calibration standards and other quality control samples are prepared and processed in exactly the same manner, and at the same time, as the samples, it is not practical for the analyst to stop an analytical run to take corrective action during the run.

11.8.2 If quality control measures do not meet the established acceptance criteria, the parts of the run between the failing quality control measures, including standards, quality control checks, and samples, must either be re-prepared and re-analyzed or the data must be qualified.

11.8.3 If a sample does not meet the established acceptance criteria, i.e. the RPD is too high or the sample failed to inject properly, that sample must be re-prepared and re-analyzed with a subsequent batch.

11.9 External verification of laboratory performance.

11.9.1 Performance evaluation (PE) samples from a third party are analyzed, if available. If the results are not within the control limits, corrective action is taken and an “Unacceptable Data for Performance Evaluation Samples” form is filled out by the analyst describing the probable error and any corrective action taken. The “Unacceptable Data” form is given to the Inorganic Chemistry Unit Supervisor, Environmental Laboratory Manager and Laboratory Quality Assurance (QA) Officer.

12.0 POLLUTION PREVENTION

12.1 For information regarding the laboratory’s pollution prevention policy and procedures see Public Health Laboratory Hazardous Waste Manual, March 17, 2006.

- 12.2 The quantity of chemicals purchased should be based on expected usage during their shelf lives, space available for storage, and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.
- 12.3 For information about pollution prevention that may be applicable to laboratory operations, consult “Less is Better: Laboratory Chemical Management to Waste Reduction” available from the American Chemical Society’s Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

13.0 WASTE MANAGEMENT

- 13.1 The Public Health Laboratory, in carrying out its mission, will do so in such a manner as to minimize pollution of the environment and manage its hazardous wastes in a safe and environmentally sound manner.
- 13.2 The Public Health Laboratory Division shall:
- Conserve natural resources through reclamation, recycling and purchasing.
 - Ensure that the Division meets all Federal, State, and Local regulations pertaining to hazardous waste disposal.
 - Prevent pollution at the source whenever possible.
 - Consider environmental impact when purchasing materials, handling chemicals and disposing of waste.
 - Promote awareness and provide training opportunities for pollution prevention and hazardous waste management within the Division.
 - Define the responsibilities of managers, supervisors and staff so that Division activities will be conducted appropriately and effectively with regard to waste management.
 - Develop policies and procedures as needed to further these objectives.
- 13.3 Follow the procedures below to avoid exposure to the contents of the drain vessel:
- 13.3.1 Use the capped plastic drain vessel provided with the instrument. Never use glass.

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- 13.3.2 Place the drain vessel on the instrument table below the peristaltic pump, where it is easy to check the liquid level.
- 13.3.3 Check the drain vessel frequently. Empty it before you ignite the plasma.
- 13.3.4 Be aware of the nature of the vessel contents. If the contents are toxic, dispose of them as hazardous waste. Also, always empty the vessel when switching from aqueous to organic sample solutions.
- 13.4 Samples containing hazardous levels of analytes should be flagged and disposed of properly.
- 13.5 For additional information regarding the laboratory's waste management policy, see Public Health Laboratory Hazardous Waste Manual, March 17, 2006.

14.0 BIBLIOGRAPHY

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- 14.4 Harrington CF, Merson SA, D'Silva TM, Method to reduce the memory effect of mercury in the analysis of fish tissue using inductively coupled plasma mass spectrometry. Anal. Chim. Acta, 2004, 505, 247-254
- 14.5 Chiou C-S, Jiang S-J, Danadurai KSK, Determination of mercury compounds in fish by microwave-assisted extraction and liquid chromatography-vapor generation inductively coupled plasma mass spectrometry. Spectrochim. Acta Part B; 2001, 56, 1133-1142

15.0 DIAGRAMS, FLOWCHARTS, VALIDATION DATA

- 15.1 The initial Demonstration of Capability data is on file; the most current MDL, precision, and accuracy data are on file in the Environmental Laboratory.

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Developed By: Zheng Yang Date: 04-16-03

Written By: Zheng Yang Date: 04-16-03

Revised By: /signed/ Betsy Edhlund Date: 03-18-11
Betsy Edhlund, Research Scientist

Approved By: /signed/ Jeffrey Brenner Date: 03-18-11
Jeffrey Brenner,
Inorganic Chemistry Unit Supervisor

Reviewed By: /signed/ Suzanne Skorich Date: 03-21-11
Suzanne Skorich, Env Lab QA Officer

Approved By: /signed/ Paul D. Swedenborg Date: 03-21-11
Paul Swedenborg,
Environmental Laboratory Section Acting Manager

Approved By: /signed/ Joanne Bartkus Date: 03-22-11
Joanne Bartkus
Public Health Laboratory Division Director



Memo

DATE: May 19, 2010

TO: Patricia McCann, Principal Investigator and Project Manager
Environmental Health Division

FROM: Suzanne Skorich, Quality Assurance Officer
Public Health Laboratory Division (PHL)

PHONE: (651) 201-5304

SUBJECT: Quality Assurance Review of Mercury Data – Surveillance report #1
Mercury Levels in Blood from Newborns in the Lake Superior Basin
GLNPO Study ID 2007-942

Nineteen sets of samples collected during the study have been analyzed for total mercury using ICP-MS. For the purposes of this review, issues relative to the quality of analysis of the samples will be discussed. Issues regarding reporting are not part of the review since sample values have not yet been reported.

The review was conducted using the acceptance criteria established by the MDH standard operating procedure (SOP) entitled “Mercury in Dried Blood Spots by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), Revision D” and the quality assurance project plan (QAPP) entitled “Mercury Levels in Blood from Newborns in the Lake Superior Basin, GLNPO ID 2007-942, Revision 2”.

Deviations from established protocols found during surveillance activities are summarized below. Included is a discussion of corrective actions taken or planned, where necessary.

Because the calibration standards and other quality control check solutions are procedural (prepared and processed in exactly the same manner, and at the same time, as the samples), it is not practical for the analyst to stop an analytical run to take corrective action during the middle of the run. Therefore, if quality control measures do not meet the established acceptance criteria, the



entire run, including standards, quality control checks, and samples, must either be re-prepared or the data qualified.

It is my judgment that 14 of the 19 sets of data met the measurement quality objectives (MQOs) defined in the SOP and/or QAPP, except where noted. The results from the following sample sets are recommended to be reported.

Sample Set	Analysis Date	Number of samples
MN-01	December 23, 2009	20
MN-02	December 23, 2009	20
MN-03	December 30, 2009	20
MN-04	December 30, 2009	20
MN-05	December 23, 2009	20
MN-06*	December 23, 2009	20
MN-07*	April 15, 2010	20
MN-08*	April 15, 2010	20
MN-09*	April 16, 2010	20
MN-10*	April 16, 2010	20
WI-01	December 29, 2009	20
WI-04	December 31, 2009	20
WI-repeat	February 23, 2010	1 (repeated analysis)
WI-05	March 26, 2010	20

* The report level verification (RLV) standard was recovered below 60% recovery. Data is recommended to be reported and qualified.

The samples from the following batches did not meet the measurement quality objectives (MQOs) of the SOP and/or QAPP and are recommended to be reanalyzed (see discussion of individual issues that follow).

Sample Set	Analysis Date	Source of MQO issues
WI-02	December 29, 2009	QCS, LCS
WI-03	March 26, 2010	LCS
WI-06	March 26, 2010	QCS
WI-07	April 13, 2010	QCS
WI-08	April 13, 2010	QCS, LCS

Sample Preservation and Sample Storage

Samples are being held frozen and in a locked freezer in a controlled access room within the laboratory. An internal chain-of-custody procedure, documenting the location of the samples at all times and in whose possession they are, has not been maintained. The laboratory has implemented the requirement since the date of this report.



Instrument Calibration, Calibration Verification (CVS), Report Level Verification (RLV)

Instrument data was reviewed to determine how the daily calibration technique of linear regression was applied and whether the acceptance criterion was met. All nineteen of the samples sets indicated a linear relationship of the standards with correlation coefficients exceeding 0.999 in all cases.

Instrument data was reviewed to determine if the periodic calibration verification steps throughout the analytical run were applied at the correct frequency. Calibration verification was not performed at the frequency of once after every 20 samples as described in the SOP. The analyst did not count the sample blanks (blank filter paper punched from each patient sample's card) as samples, even though they occupied positions in the 96-well plate and were injected as samples into the ICP-MS. The analyst will add an additional set of calibration verification standards after 20 sample or method blank injections in all future runs.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the calibration verification steps throughout the analytical run. All data passed the acceptance criteria.

Finally, instrument data was reviewed to determine whether the two checks on low level quantitation at the reporting level of 2.42 ug/L total mercury met the criterion. The following table summarizes data sets for which the report level verification (RLV) checks have not passed the acceptance criterion of 60-140% recovery. Data for samples in the associated sample sets are recommended to be reported; however the patient samples in these sets are recommended to be qualified to indicate that the RLV did not meet the requirements of the QAPP.

Sample Set	Analysis Date	Reason for QC failure
MN-06	April 14, 2010	50% < 60% recovery
MN-07	April 15, 2010	57% < 60% recovery
MN-08	April 15, 2010	49% < 60% recovery
MN-09	April 16, 2010	53% < 60% recovery
MN-10	April 16, 2010	57% < 60% recovery
WI-08	April 13, 2010	47% < 60% recovery

Data collected through the course of the study indicates that the RLV criteria can be met when the instrument conditions are optimized. In April, the instrument was not operating under optimal conditions causing insufficient sensitivity at low concentrations.

Maintenance on the ICP-MS was performed in the early weeks of May to optimize performance. The maintenance included cleaning the sample introduction components and optimizing detector voltages, which seemed to restore the sensitivity of the instrument that appeared to have dropped in the first part of April.



The laboratory is committed to monitoring the instrument more carefully and taking preventive action (cleaning the sample introduction components, optimizing detector voltages, etc.) more frequently as the study progresses in order to achieve acceptable quantitation at the RL.

Holding Time

Holding times have not been established for the method. Since all samples are being held in a freezer, significant degradation of mercury is not expected. There are no deviations from protocol to report on holding times.

QC Accuracy and Precision: Laboratory Control Spikes (LCS), Laboratory Control Spike Duplicates (LCSD), and Quality Control Samples (QCS)

Blank spike (LCS & LCSD) data and certified reference material (QCS) data were reviewed to determine if any recoveries were less 80% or greater than 120%. The analytical runs for the sample sets listed below are deemed to not be in statistical control because of LCS and QCS failures and are not to be reported. The samples sets will be reanalyzed at some time during the course of the study. Since there is sufficient sample to analyze these samples only once more, the analyst will make sure that the instrument conditions are optimized prior to reanalyzing these samples.

Sample Set	Reason for LCS failure	Reason for LCSD failure	Reason for QCS failure
WI-02	End of run < 80%		End of run < 80%
WI-03	End of run not analyzed due to lab accident	Could not evaluate precision because end of run's lab accident	
WI-06			Beginning of run >120%
WI-07			Beginning of run < 80% and End of run < 80%
WI-08	End of run < 80%		Beginning of run < 80% and End of run < 80%



Field Duplicates

All sample sets contained field duplicates. The concentrations of ten of the 19 sets of field duplicates were below the method detection limit (MDL). The remaining nine sets met the requirements for precision (results within +/- RL when the calculated concentration is < 10 times the MDL).

Aqueous Blanks

Instrument data was reviewed to determine if the evaluation of low system background using aqueous blanks (Abs) throughout the analytical run were applied at the correct frequency. Aqueous blank analysis was not performed at the frequency of once after every 20 samples as described in the SOP. As with the calibration verification standards, the analyst did not count the sample blanks (blank filter paper punched from each patient sample's card) as samples, even though they occupied positions in the 96-well plate and were injected as samples into the ICP-MS. The analyst will add the appropriate aqueous blanks to all future runs.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the aqueous blanks throughout the analytical run. The following table summarizes data for which the aqueous blank analyzed immediately after calibration has not passed the acceptance criteria. In all four instances, the AB that followed immediately after the initial AB was below the MDL. In my opinion, data need not be qualified based on this QC failure.

Sample Set	Analysis Date	Reason for QC failure
MN-01	December 23, 2009	> MDL; next AB passed
MN-03	December 30, 2009	> MDL; next AB passed
WI-02	December 29, 2009	> MDL; next AB passed
WI-03	March 26, 2010	> MDL; next AB passed

Method Blanks

All sample sets contained a method blank (MB) corresponding to each patient sample in the set. No instance was noted where a MB was found to be greater than the MDL while the corresponding patient sample exceeded the reporting limit (RL). Seven instances were noted where a MB was found to be greater than the MDL while the corresponding patient sample was also estimated to be between the MDL and the RL. In my opinion, the patient data should be qualified at the time it is reported to indicate that an estimated value was also found in the MB for that sample.



Other Issues

The laboratory's SOP indicates that the internal standard is rhodium; however, the analyst has always used iridium because of the proximity of the quantitation mass to that of mercury. The laboratory's SOP will be rewritten to rectify this error.

Errors in documenting standards preparation prevented me from determining the traceability and concentration of the standard reference materials in use, except by inference, because key steps in the documentation process were omitted. The analyst has agreed to maintain accurate records on all sample preparation and standard preparation steps in the future.

Laboratory notebooks and forms have not been signed and dated by an equally competent reviewer. The Inorganic Unit supervisor has agreed to review the analyst's work more frequently and to sign and date the notebook and forms as they are reviewed.

Maintenance on the ICP-MS was performed in the early weeks of May to optimize performance. The maintenance included cleaning the sample introduction components and optimizing detector voltages, which seemed to restore the sensitivity of the instrument that appeared to have dropped in the first part of April. The analyst, supervisor, and I are confident that sample analysis can again proceed; however, we will monitor the system performance more closely. In order to monitor the progress, we have agreed that fewer batches of samples should be set up each day during the next few weeks.

Please let me know if you have any questions or concerns with the issues in this report or any other issues regarding the laboratory's role in the study.

SSS

cc: Paul Swedenborg, Acting Environmental Laboratory Manager, PHL



Memo

DATE: July 2, 2010

TO: Patricia McCann, Principal Investigator and Project Manager
Environmental Health Division

FROM: Suzanne Skorich, Quality Assurance Officer
Public Health Laboratory Division (PHL)

PHONE: (651) 201-5304

SUBJECT: Quality Assurance Review of Mercury Data – Surveillance report #2
Mercury Levels in Blood from Newborns in the Lake Superior Basin
GLNPO Study ID 2007-942

Twenty six sets of samples collected during the study have been analyzed for total mercury using ICP-MS. Seven sets of samples have been analyzed since I sent you Surveillance report #1, dated May 19, 2010. For the purposes of this review, issues relative to the quality of analysis of the most recent seven sample sets will be discussed. Issues regarding reporting are not part of the review since sample values have not yet been reported.

The review was conducted using the acceptance criteria established by the MDH standard operating procedure (SOP) entitled “Mercury in Dried Blood Spots by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), Revision D” and the quality assurance project plan (QAPP) entitled “Mercury Levels in Blood from Newborns in the Lake Superior Basin, GLNPO ID 2007-942, Revision 2”.

Deviations from established protocols found during surveillance activities conducted since May 19, 2010 are summarized below. Included is a discussion of corrective actions taken or planned, where necessary.

Because the calibration standards and other quality control check solutions are procedural (prepared and processed in exactly the same manner, and at the same time, as the samples), it is not practical for the analyst to stop an analytical run to take corrective action during the middle of



the run. Therefore, if quality control measures do not meet the established acceptance criteria, the parts of the run between quality control measures, including standards, quality control checks, and samples, must either be re-prepared or the data qualified.

In addition to reporting on the seven sets of samples analyzed since May 19, 2010, outstanding issues from Surveillance report #1 will be discussed.

It is my judgment that all sets of data discussed in this report met the measurement quality objectives (MQOs) defined in the SOP and/or QAPP, except where noted. The results from the following sample sets are recommended to be reported.

Sample Set	Analysis Date	Number of samples
MN-11	June 8, 2010	20
MN-12	June 10, 2010	10 (see below)
MN-14	June 16, 2010	20
MN-15	June 17, 2010	20
MN-16	June 18, 2010	20
MN-17	June 22, 2010	20

The samples from the following batches did not meet the measurement quality objectives (MQOs) of the SOP and/or QAPP and are recommended to be reanalyzed (see discussion of individual issues that follow).

Sample Set	Analysis Date	Source of MQO issues
MN-12	June 10, 2010	LCS final
MN-13	June 11, 2010	LCS mid-run

Sample Preservation and Sample Storage

There are no deviations from protocol to report on sample preservation and sample storage.

Instrument Calibration, Calibration Verification (CVS), Report Level Verification (RLV)

Instrument data was reviewed to determine how the daily calibration technique of linear regression was applied and whether the acceptance criterion was met. All seven of the samples sets indicated a linear relationship of the standards with correlation coefficients exceeding 0.999 in all cases.

Instrument data was reviewed to determine if the periodic calibration verification steps throughout the analytical run were applied at the correct frequency. Calibration verification is performed at the frequency of once after every 20 samples as described in the SOP.



Instrument data was reviewed to determine whether the acceptance criteria were met for all of the calibration verification steps throughout the analytical run. All data passed the acceptance criteria.

Finally, instrument data was reviewed to determine whether the check on low level quantitation at the reporting level (RLV) of 2.42 ug/L total mercury met the criterion. All seven of the checks met the 60-140% recovery criterion as shown below.

Sample Set	Analysis Date	RLV Recovery
MN-11	June 8, 2010	94%
MN-12	June 10, 2010	92%
MN-13	June 11, 2010	86%
MN-14	June 16, 2010	78%
MN-15	June 17, 2010	92%
MN-16	June 18, 2010	86%
MN-17	June 22, 2010	82%

As noted in Surveillance report #1, the instrument must operate under optimal conditions to achieve sensitivity at low concentrations. The analyst has been dutiful in taking preventive action (cleaning the sample introduction components, optimizing detector voltages, etc.) more frequently as the study progresses in order to achieve acceptable quantitation at the RL. Improved performance of the RLV is an indicator that the preventive actions have had a positive effect on the measurement system.

Holding Time

Holding times have not been established for the method. Since all samples are being held in a freezer, significant degradation of mercury is not expected. There are no deviations from protocol to report on holding times.

QC Accuracy and Precision: Laboratory Control Spikes (LCS), Laboratory Control Spike Duplicates (LCSD), and Quality Control Samples (QCS)

Blank spike (LCS & LCSD) data and certified reference material (QCS) data were reviewed to determine if any recoveries were less 80% or greater than 120%.

The analytical runs for the sample sets listed below contained LCS and QCS failures (below 80% recovery) and are not to be reported. The samples sets will be reanalyzed at some time during the course of the study. Since there is sufficient sample to analyze these samples only once more, the analyst will make sure that the instrument conditions are optimized prior to reanalyzing these samples.

Sample Set	Reason for LCS failure	Reason for LSCD failure	Reason for QCS failure
MN-12	End of run < 80%		
MN-13	Mid-run < 80%	Mid-run > 20 RPD	

The laboratory has thus far collected fifty nine data points for the QCS and fifty eight data points for the LCS. The following table summarizes the recoveries and control limits that are derived from the data collected.

QC Measure	True Value	N	Mean Recovery	Statistically-derived control limits (mean $\pm$ 3 std dev) calculated from all N	SOP-designated control limits
QCS (SRM 966, bovine blood)	31.4 ug/L	59	91%	65 – 118 %	80 – 120 %
LCS (SRM 966, bovine blood spotted on card)	31.4 ug/L	58	88%	72 – 104%	80 – 120 %

It is my opinion that the data indicates that it is more difficult to extract mercury from blood than we originally predicted when the laboratory wrote its SOP in which we stated that the control limits should be recalculated as data is collected, but that the limits should not be wider than 80-120% recovery.

Of the twenty six sets of samples analyzed to date, six were rejected based on QCS and LCS recoveries being < 80%. The highlighted recoveries illustrate how difficult it is to meet the 80% criterion and also illustrate that the values, while rejected based on the fixed limits, are well within the statistically-derived control limits generated from data collected during the study.

Sample Set	LCS recoveries (beginning and end)	LSCD RPD (criterion < 20%)	QCS recoveries (beginning and end)
WI-02	90% and 78%	15%	83% and 76%
WI-03	85% and lab accident	Lab accident	116% and 110%
WI-07	91% and 93%	2%	78% and 77%
WI-08	80% and 77%	4%	78% and 70%
MN-12 – second set	89% and 79%	13%	91% and 91%
MN-13 – first set	99% and 78%	24%	105% and 99%
MN-13 – second set	78% and 84%	7%	99% and 100%

The laboratory plans to reanalyze all of the sample sets; however, we wish you to be aware that it is likely that reanalysis will result in data that doesn't conform to the QCS and LCS acceptance



criteria we have established. At that time, because we will have used up all of the available blood spots, if both the initial run and the repeat run are flawed, it may be necessary to choose one set over the other and to qualify the data.

Field Duplicates

All sample sets contained field duplicates. The concentrations of five of the seven sets of field duplicates were below the method detection limit (MDL). The remaining two sets met the requirements for precision (results within +/- RL when the calculated concentration is < 10 times the MDL).

Aqueous Blanks

Instrument data was reviewed to determine if the evaluation of low system background using aqueous blanks (AB) throughout the analytical run were applied at the correct frequency. Aqueous blank analysis are performed at the frequency of once after every 20 samples as described in the SOP.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the aqueous blanks throughout the analytical run. The following table summarizes data for which the aqueous blank analyzed immediately after calibration did not pass the acceptance criteria. In all instances, the AB that followed immediately after the initial AB was below the MDL. In my opinion, data need not be qualified based on this QC failure.

Sample Set	Analysis Date	Reason for QC failure
MN-11	June 8, 2010	> MDL; next AB passed
MN-12	June 10, 2010	> MDL; next AB passed
MN-14	June 16, 2010	> MDL; next AB passed

Method Blanks

All sample sets contained a method blank (MB) corresponding to each patient sample in the set. No instance was noted where a MB was found to be greater than the MDL while the corresponding patient sample exceeded the reporting limit (RL). Two instances were noted where a MB was found to be greater than the MDL while the corresponding patient sample was below the MDL. In my opinion, the patient data should be qualified at the time it is reported to indicate that an estimated value was found in the MB for that sample.



Other Issues

The laboratory's SOP has not been revised to indicate that the internal standard is iridium, not rhodium.

Records of sample preparation and standard preparation steps have greatly improved. Standard preparation was completely traceable in the seven sets of data under review.

Laboratory notebooks and forms will be signed and dated by the quality assurance officer as they are reviewed.

Please let me know if you have any questions or concerns with the issues in this report or any other issues regarding the laboratory's role in the study.

SSS

cc: Paul Swedenborg, Acting Environmental Laboratory Manager, PHL



Memo

DATE: August 6, 2010

TO: Patricia McCann, Principal Investigator and Project Manager
Environmental Health Division

FROM: Suzanne Skorich, Quality Assurance Officer
Public Health Laboratory Division (PHL)

PHONE: (651) 201-5304

SUBJECT: Quality Assurance Review of Mercury Data – Surveillance report #3
Mercury Levels in Blood from Newborns in the Lake Superior Basin
GLNPO Study ID 2007-942

Thirty six sets of samples collected during the study have been analyzed for total mercury using ICP-MS. Ten sets of samples have been analyzed since I sent you Surveillance report #2, dated July 2, 2010. For the purposes of this review, issues relative to the quality of analysis of the most recent ten sample sets will be discussed. Issues regarding reporting are not part of the review since sample values have not yet been reported.

The review was conducted using the acceptance criteria established by the MDH standard operating procedure (SOP) entitled "Mercury in Dried Blood Spots by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), Revision D" and the quality assurance project plan (QAPP) entitled "Mercury Levels in Blood from Newborns in the Lake Superior Basin, GLNPO ID 2007-942, Revision 2".

Deviations from established protocols found during surveillance activities conducted since July 2, 2010 are summarized below. Included is a discussion of corrective actions taken or planned, where necessary.

Because the calibration standards and other quality control check solutions are procedural (prepared and processed in exactly the same manner, and at the same time, as the samples), it is not practical for the analyst to stop an analytical run to take corrective action during the middle of



the run. Therefore, if quality control measures do not meet the established acceptance criteria, the parts of the run between quality control measures, including standards, quality control checks, and samples, must either be re-prepared or the data qualified.

In addition to reporting on the ten sets of samples analyzed since May 19, 2010, outstanding issues from Surveillance reports #1 and #2 will be discussed.

It is my judgment that nine sets of data discussed in this report met the measurement quality objectives (MQOs) defined in the SOP and/or QAPP, except where noted. The results from the following sample sets are recommended to be reported.

Sample Set	Analysis Date	Number of samples
MN-18	June 23, 2010	20
MN-20	July 2, 2010	20
MN-21	July 7, 2010	20
MN-22	July 8, 2010	20
MN-23	July 9, 2010	20
MN-24	July 13, 2010	20
MN-25	July 14, 2010	20
MN-26	July 15, 2010	20
MN-27	July 16, 2010	10 (see below)

The samples from the following batches did not meet the measurement quality objectives (MQOs) of the SOP and/or QAPP and are recommended to be reanalyzed (see discussion of individual issues that follow).

Sample Set	Analysis Date	Source of MQO issues
MN-19	June 24, 2010	Calibration not verified
MN-27	July 16, 2010	LCS final (79% < 80%)

Sample Preservation and Sample Storage

There are no deviations from protocol to report on sample preservation and sample storage.

Instrument Calibration, Calibration Verification (CVS), Report Level Verification (RLV)

Instrument data was reviewed to determine how the daily calibration technique of linear regression was applied and whether the acceptance criterion was met. All ten of the samples sets indicated a linear relationship of the standards with correlation coefficients exceeding 0.999 in all cases.



Instrument data was reviewed to determine if the periodic calibration verification steps throughout the analytical run were applied at the correct frequency. Calibration verification is performed at the frequency of once after every 20 samples as described in the SOP.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the calibration verification steps throughout the analytical run. All data passed the acceptance criteria, except for that of batch MN-19 which was analyzed on June 24, 2010. In this analytical run, the last two of the three low level calibration verification standards and the last one of the three medium level calibration verification standards were outside of the 2-sigma control limits and both were on the low side of the mean.

The analyst promptly took corrective action by stopping further analysis of patient samples and evaluating the calibration verification standards against newly-prepared standards. No significant difference was noted between the newly-prepared standards and the ones in use on June 24th. The analyst performed routine optimization for the instrument, but found no factor that could be attributed to the low responses observed on June 24th. The subsequent analytical batches passed the calibration verification criteria using the same standards that were in use on June 24th.

Finally, instrument data was reviewed to determine whether the check on low level quantitation at the reporting level (RLV) of 2.42 ug/L total mercury met the criterion. All nine of the checks in the batches that are recommended for reporting met the 60-140% recovery criterion as shown below.

Sample Set	Analysis Date	RLV Recovery
MN-18	June 23, 2010	83%
MN-20	July 2, 2010	93%
MN-21	July 7, 2010	116%
MN-22	July 8, 2010	76%
MN-23	July 9, 2010	88%
MN-24	July 13, 2010	74%
MN-25	July 14, 2010	74%
MN-26	July 15, 2010	86%
MN-27	July 16, 2010	79%

Holding Time

Holding times have not been established for the method. Since all samples are being held in a freezer, significant degradation of mercury is not expected. There are no deviations from protocol to report on holding times.



QC Accuracy and Precision: Laboratory Control Spikes (LCS), Laboratory Control Spike Duplicates (LCSD), and Quality Control Samples (QCS)

Blank spike (LCS & LCSD) data and certified reference material (QCS) data were reviewed to determine if any recoveries were less 80% or greater than 120%.

The analytical runs for the sample sets listed below contained LCS and QCS failures (below 80% recovery) and are not to be reported. The samples sets will be reanalyzed at some time during the course of the study. Since there is sufficient sample to analyze these samples only once more, the analyst will make sure that the instrument conditions are optimized prior to reanalyzing these samples.

Sample Set	Reason for LCS failure	Reason for LCSD failure	Reason for QCS failure
MN-27	Final < 80%		

Adding to the data provided in Surveillance report #2, the laboratory has thus far collected 107 data points for the QCS and 106 data points for the LCS. The following table summarizes the recoveries and control limits that are derived from the data collected.

QC Measure	True Value	N	Mean Recovery	Statistically-derived control limits (mean $\pm$ 3 std dev) calculated from all N	SOP-designated control limits
QCS (SRM 966, bovine blood)	31.4 ug/L	107	95%	86 – 105 %	80 – 120 %
LCS (SRM 966, bovine blood spotted on card)	31.4 ug/L	106	89%	70 – 109%	80 – 120 %

As stated above, the final LCS in batch MN-27 was recovered at 79%, failing to meet the 80-120% criterion, but it was within the statistically-derived control limits of 70-109%.

The laboratory plans to reanalyze the last 10 samples in batch MN-27; however, we wish you to be aware that it is likely that reanalysis will result in data that doesn't conform to the QCS and LCS acceptance criteria we have established. At that time, because we will have used up all of the available blood spots, if both the initial run and the repeat run are flawed, it may be necessary to choose one set over the other and to qualify the data.



Field Duplicates

All sample sets contained field duplicates. All nine sets of field duplicates were above the method detection limit (MDL), with seven sets showing results between the MDL and the reporting limit (RL). Results between the MDL and the RL are qualified with a "J" signifying estimated concentrations. The remaining two sets consisted of samples with results above the RL and also with concentrations > 10 times the MDL.

The seven sets of duplicates with results that are qualified as estimated concentration ("J-flagged") met the requirements for precision (results within +/- RL when the calculated concentration is < 10 times the MDL).

The two sets of duplicates with results that are > RL met the requirements for precision (results with relative percent difference (RPD) of less than or equal to 20 when the calculated concentration is < 10 times the MDL).

Aqueous Blanks

Instrument data was reviewed to determine if the evaluation of low system background using aqueous blanks (AB) throughout the analytical run were applied at the correct frequency. Aqueous blank analysis are performed at the frequency of once after every 20 samples as described in the SOP.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the aqueous blanks throughout the analytical run. All AB samples passed the acceptance criteria.

Method Blanks

All sample sets contained a method blank (MB) corresponding to each patient sample in the set. No instance was noted where a MB was found to be greater than the MDL while the corresponding patient sample exceeded the reporting limit (RL).

Other Issues

The laboratory's SOP has not been revised to indicate that the internal standard is iridium, not rhodium.

Records of sample preparation and standard preparation steps have greatly improved. Standard preparation was completely traceable in the ten sets of data under review.



Laboratory notebooks and forms are now signed and dated by the quality assurance officer as they are reviewed.

Please let me know if you have any questions or concerns with the issues in this report or any other issues regarding the laboratory's role in the study.

SSS

cc: Betsy Edhlund, Research Scientist 2, PHL
Jeff Brenner, Inorganic Chemistry Unit Supervisor, PHL
Paul Swedenborg, Acting Environmental Laboratory Manager, PHL



Memo

DATE: September 24, 2010

TO: Patricia McCann, Principal Investigator and Project Manager
Environmental Health Division

FROM: Suzanne Skorich, Quality Assurance Officer
Public Health Laboratory Division (PHL)

PHONE: (651) 201-5304

SUBJECT: Quality Assurance Review of Mercury Data – Surveillance report #4
Mercury Levels in Blood from Newborns in the Lake Superior Basin
GLNPO Study ID 2007-942

Forty six sets of samples collected during the study have been analyzed for total mercury using ICP-MS. Ten sets of samples have been analyzed since I sent you Surveillance report #3, dated August 6, 2010. For the purposes of this review, issues relative to the quality of analysis of the most recent ten sample sets will be discussed. Issues regarding reporting are not part of the review since sample values have not yet been reported.

The review was conducted using the acceptance criteria established by the MDH standard operating procedure (SOP) entitled “Mercury in Dried Blood Spots by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), Revision D” and the quality assurance project plan (QAPP) entitled “Mercury Levels in Blood from Newborns in the Lake Superior Basin, GLNPO ID 2007-942, Revision 2”.

Deviations from established protocols found during surveillance activities conducted since August 6, 2010 are summarized below. Included is a discussion of corrective actions taken or planned, where necessary.

Because the calibration standards and other quality control check solutions are procedural (prepared and processed in exactly the same manner, and at the same time, as the samples), it is not practical for the analyst to stop an analytical run to take corrective action during the middle of



the run. Therefore, if quality control measures do not meet the established acceptance criteria, the parts of the run between quality control measures, including standards, quality control checks, and samples, must either be re-prepared or the data qualified.

In addition to reporting on the ten sets of samples analyzed since July 27, 2010, outstanding issues from Surveillance reports #1 - #3 will be discussed.

It is my judgment that all ten sets of data discussed in this report met the measurement quality objectives (MQOs) defined in the SOP and/or QAPP, except where noted. The results from the following sample sets are recommended to be reported.

Sample Set	Analysis Date	Number of samples
MN-28	July 27, 2010	20
MN-29	July 28, 2010	20
MN-30	July 29, 2010	20
MN-31	July 30, 2010	20
MN-32	August 3, 2010	20
MN-33	August 4, 2010	20
MN-34	August 5, 2010	20
MN-35	August 6, 2010	20
MN-36	August 10, 2010	20
MN-37	August 11, 2010	19 (see below)

Sample Preservation and Sample Storage

There are no deviations from protocol to report on sample preservation and sample storage.

Instrument Calibration, Calibration Verification (CVS), Report Level Verification (RLV)

Instrument data was reviewed to determine how the daily calibration technique of linear regression was applied and whether the acceptance criterion was met. All ten of the samples sets indicated a linear relationship of the standards with correlation coefficients exceeding 0.999 in all cases.

Instrument data was reviewed to determine if the periodic calibration verification steps throughout the analytical run were applied at the correct frequency. Calibration verification is performed at the frequency of once after every 20 samples as described in the SOP.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the calibration verification steps throughout the analytical run. All data passed the acceptance criteria.



Finally, instrument data was reviewed to determine whether the check on low level quantitation at the reporting level (RLV) of 2.42 ug/L total mercury met the criterion. All ten of the checks in the batches met the 60-140% recovery criterion as shown below.

Sample Set	Analysis Date	RLV Recovery
MN-28	July 27, 2010	74%
MN-29	July 28, 2010	73%
MN-30	July 29, 2010	72%
MN-31	July 30, 2010	92%
MN-32	August 3, 2010	88%
MN-33	August 4, 2010	82%
MN-34	August 5, 2010	71%
MN-35	August 6, 2010	88%
MN-36	August 10, 2010	83%
MN-37	August 11, 2010	88%

Holding Time

Holding times have not been established for the method. Since all samples are being held in a freezer, significant degradation of mercury is not expected. There are no deviations from protocol to report on holding times.

QC Accuracy and Precision: Laboratory Control Spikes (LCS), Laboratory Control Spike Duplicates (LCSD), and Quality Control Samples (QCS)

Blank spike (LCS & LCSD) data and certified reference material (QCS) data were reviewed to determine if any recoveries were less 80% or greater than 120%. All LCS, LCSD, and QCS recoveries were within the limits. Precision of the LCS and LCSD in all cases was within the limit of 20% relative percent difference.



Adding to the data provided in Surveillance report #3, the laboratory has thus far collected 137 data points for the QCS and 136 data points for the LCS. The following table summarizes the recoveries and control limits that are derived from the data collected.

QC Measure	True Value	N	Mean Recovery	Statistically-derived control limits (mean $\pm$ 3 std dev) calculated from all N	SOP-designated control limits
QCS (SRM 966, bovine blood)	31.4 ug/L	137	95%	83 – 107 %	80 – 120 %
LCS (SRM 966, bovine blood spotted on card)	31.4 ug/L	136	92%	73 – 111%	80 – 120 %

Field Duplicates

All sample sets contained field duplicates. Nine of ten sets of field duplicates were above the method detection limit (MDL), with five sets showing results between the MDL and the reporting limit (RL). Results between the MDL and the RL are qualified with a “J” signifying estimated concentrations. The remaining four sets consisted of samples with results above the RL, with two sets having concentrations > 10 times the MDL.

The five sets of duplicates with results that are qualified as estimated concentration (“J-flagged”) met the requirements for precision (results within +/- RL when the calculated concentration is < 10 times the MDL).

The two sets of duplicates with results that are > RL, but with concentrations < 10times the MDL met the requirements for precision (results within +/- RL when the calculated concentration is < 10 times the MDL).

One of the two sets of duplicates (batch MN-28) with results that are > RL and with concentrations > 10times the MDL met the requirements for precision. The other set of duplicates (batch MN-31) had an RPD value of 21%, exceeding the limit of 20%.

The data for batch MN-31 shall be qualified to indicate that the RPD value of the duplicates was not within the acceptance limits of the method.



Aqueous Blanks

Instrument data was reviewed to determine if the evaluation of low system background using aqueous blanks (AB) throughout the analytical run were applied at the correct frequency. Aqueous blank analysis are performed at the frequency of once after every 20 samples as described in the SOP.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the aqueous blanks throughout the analytical run. All AB samples passed the acceptance criteria.

Method Blanks

All sample sets contained a method blank (MB) corresponding to each patient sample in the set.

One instance was noted where a MB was found to be greater than the MDL. The sample analysis showed that the internal standard did not meet the requirements of the method. Therefore, samples MN-0734 (analyzed in batch MN-37) and its associated blank will be reanalyzed.

Other Issues

Ten patient samples were observed to have concentrations of total mercury that exceeded the concentration of the highest standard in the calibration curve. All but one sample was below the known range of linearity of 242 ug/L total mercury. The samples with values that are outside of the calibration range, but within the known linearity range, are listed below. Results for these samples will be reported as "estimated." One sample, MN-0733 is slightly higher than the known linear range of the instrument. The result will also be reported and qualified as "estimated."

Sample Number	Batch Number	Analysis Date	Reported Value (ug/L total mercury)	Data Qualifier
MN-0544	MN-28	July 27, 2010	89	Estimated result; reported value is over the calibration range but within the known linear range.
MN-0545	MN-28	July 27, 2010	80	
MN-0560	MN-28	July 27, 2010	83	
MN-0587	MN-30	July 29, 2010	75	
MN-0625	MN-32	August 3, 2010	128	
MN-0635	MN-32	August 3, 2010	63	
MN-0698	MN-35	August 6, 2010	191	
MN-0703	MN-36	August 10, 2010	54	
MN-0719	MN-36	August 10, 2010	201	
MN-0733	MN-37	August 11, 2010	247	Estimated result; reported value is over the known linear range.



The laboratory's SOP has not been revised to indicate that the internal standard is iridium, not rhodium.

Please let me know if you have any questions or concerns with the issues in this report or any other issues regarding the laboratory's role in the study.

SSS

cc: Betsy Edhlund, Research Scientist 2, PHL
Jeff Brenner, Inorganic Chemistry Unit Supervisor, PHL
Paul Swedenborg, Acting Environmental Laboratory Manager, PHL



Memo

DATE: October 6, 2010

TO: Patricia McCann, Principal Investigator and Project Manager
Environmental Health Division

FROM: Suzanne Skorich, Quality Assurance Officer
Public Health Laboratory Division (PHL)

PHONE: (651) 201-5304

SUBJECT: Quality Assurance Review of Mercury Data – Surveillance report #5
Mercury Levels in Blood from Newborns in the Lake Superior Basin
GLNPO Study ID 2007-942

Sixty sets of samples collected during the study have been analyzed for total mercury using ICP-MS. Fourteen sets of samples have been analyzed since I sent you Surveillance report #4, dated September 24, 2010. For the purposes of this review, issues relative to the quality of analysis of the most recent fourteen sample sets will be discussed. Issues regarding reporting are not part of the review since sample values have not yet been reported.

The review was conducted using the acceptance criteria established by the MDH standard operating procedure (SOP) entitled “Mercury in Dried Blood Spots by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), Revision D” and the quality assurance project plan (QAPP) entitled “Mercury Levels in Blood from Newborns in the Lake Superior Basin, GLNPO ID 2007-942, Revision 2”.

Deviations from established protocols found during surveillance activities conducted since September 24, 2010 are summarized below. Included is a discussion of corrective actions taken or planned, where necessary.

Because the calibration standards and other quality control check solutions are procedural (prepared and processed in exactly the same manner, and at the same time, as the samples), it is not practical for the analyst to stop an analytical run to take corrective action during the middle of



the run. Therefore, if quality control measures do not meet the established acceptance criteria, the parts of the run between quality control measures, including standards, quality control checks, and samples, must either be re-prepared or the data qualified.

In addition to reporting on the ten sets of samples analyzed since August 11, 2010, outstanding issues from Surveillance reports #1 - #4 will be discussed.

It is my judgment that twelve sets of data discussed in this report met the measurement quality objectives (MQOs) defined in the SOP and/or QAPP, except where noted. The results from the following sample sets are recommended to be reported.

Sample Set	Analysis Date	Number of samples
MN-38	August 12, 2010	18 (see below)
MN-39	August 13, 2010	20
MN-40	August 17, 2010	20
MN-41	August 18, 2010	20
MN-42	August 19, 2010	20
MN-44	August 25, 2010	10 (see below)
MN-45	August 31, 2010	20
MN-46	September 1, 2010	10 (see below)
MN-47	September 2, 2010	20
MN-48	September 3, 2010	20
MN-49	September 8, 2010	20
MN-50	September 9, 2010	10 (see below)

The samples from the following batches did not meet the measurement quality objectives (MQOs) of the SOP and/or QAPP and are recommended to be reanalyzed (see discussion of individual issues that follow).

Sample Set	Analysis Date	Source of MQO issues
MN-38	August 12, 2010	Two sample solutions (MN-0747 and MN-0760 Dup) ran out prior to analysis completion due to an instrument error
MN-43	August 20, 2010	All QCS samples > 120% recovery; first pair LCS precision > 20%
MN-44	August 25, 2010	Calibration not verified because final low CVS failed to inject; LCS final (78%<80%)
MN-46	September 1, 2010	LCS initial (79%<80%)
MN-50	September 9, 2010	Calibration not verified because final high CVS failed to inject
MN-51	September 10, 2010	Calibration not verified; LCS failed precision and accuracy checks due to lab accident at the time of sample preparation



Sample Preservation and Sample Storage

There are no deviations from protocol to report on sample preservation and sample storage.

Instrument Calibration, Calibration Verification (CVS), Report Level Verification (RLV)

Instrument data was reviewed to determine how the daily calibration technique of linear regression was applied and whether the acceptance criterion was met. All ten of the samples sets indicated a linear relationship of the standards with correlation coefficients exceeding 0.999 in all cases.

Instrument data was reviewed to determine if the periodic calibration verification steps throughout the analytical run were applied at the correct frequency. Calibration verification is performed at the frequency of once after every 20 samples as described in the SOP.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the calibration verification steps throughout the analytical run. All data passed the acceptance criteria.

Finally, instrument data was reviewed to determine whether the check on low level quantitation at the reporting level (RLV) of 2.42 ug/L total mercury met the criterion. All fourteen of the checks in the batches met the 60-140% recovery criterion as shown below.

Sample Set	Analysis Date	RLV Recovery
MN-38	August 12, 2010	78%
MN-39	August 13, 2010	68%
MN-40	August 17, 2010	94%
MN-41	August 18, 2010	68%
MN-42	August 19, 2010	86%
MN-43	August 20, 2010	89%
MN-44	August 25, 2010	82%
MN-45	August 31, 2010	88%
MN-46	September 1, 2010	80%
MN-47	September 2, 2010	81%
MN-48	September 3, 2010	73%
MN-49	September 8, 2010	86%
MN-50	September 9, 2010	73%
MN-51	September 10, 2010	81%

Holding Time

Holding times have not been established for the method. Since all samples are being held in a freezer, significant degradation of mercury is not expected. There are no deviations from protocol to report on holding times.

QC Accuracy and Precision: Laboratory Control Spikes (LCS), Laboratory Control Spike Duplicates (LCSD), and Quality Control Samples (QCS)

Blank spike (LCS & LCSD) data and certified reference material (QCS) data were reviewed to determine if any recoveries were less 80% or greater than 120%. Except for batches MN-43, MN-44, MN-46, and MN-51, the LCS, LCSD, and QCS recoveries were within the 80-120% limits. Precision of the LCS and LCSD in all cases, except in MN-43 and MN-51, was within the limit of 20% relative percent difference.

Adding to the data provided in Surveillance report #4, the laboratory has thus far collected 176 data points for the QCS and 177 data points for the LCS. Four outliers were identified and removed from the dataset prior to determining the statistically-derived control limits. The following table summarizes the recoveries and control limits that are derived from the data collected.

QC Measure	True Value	N	Mean Recovery	Statistically-derived control limits (mean $\pm$ 3 std dev) calculated from all N	SOP-designated control limits
QCS (SRM 966, bovine blood)	31.4 ug/L	179	94%	84 – 104 %	80 – 120 %
LCS (SRM 966, bovine blood spotted on card)	31.4 ug/L	178	90%	72 – 107%	80 – 120 %

Field Duplicates

All sample sets contained field duplicates. Six of fourteen sets of field duplicates were above the method detection limit (MDL), with two sets showing results between the MDL and the reporting limit (RL). Results between the MDL and the RL are qualified with a “J” signifying estimated concentrations. The remaining four sets consisted of samples with results above the RL, with two sets having concentrations > 10 times the MDL.

The two sets of duplicates with results that are qualified as estimated concentration (“J-flagged”) met the requirements for precision (results within +/- RL when the calculated concentration is < 10 times the MDL).



The two sets of duplicates with results that are $> RL$, but with concentrations < 10 times the MDL met the requirements for precision (results within $\pm RL$ when the calculated concentration is < 10 times the MDL).

One of the two sets of duplicates (batch MN-38) with results that are $> RL$ and with concentrations > 10 times the MDL met the requirements for precision.

The other set of field duplicates from batch MN-51 are not reported because the batch did not meet the acceptance criteria for calibration and other quality control determinations due to laboratory accident at the time of sample preparation.

Aqueous Blanks

Instrument data was reviewed to determine if the evaluation of low system background using aqueous blanks (AB) throughout the analytical run were applied at the correct frequency. Aqueous blank analysis are performed at the frequency of once after every 20 samples as described in the SOP.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the aqueous blanks throughout the analytical run. All AB samples passed the acceptance criteria.

Method Blanks

All sample sets contained a method blank (MB) corresponding to each patient sample in the set.

Three instances were noted where a MB was found to be greater than the MDL. In two instances the blank indicated a result between the MDL and the RL (marked "J" as estimated concentrations) while the corresponding two samples were found to be $< RL$. Sample results will be reported as below the RL.

The other positive MB corresponds with patient sample MN-0836. Since the concentration of total mercury in the MN-0836 was over six times that found in the MB, the sample result will be reported and qualified to indicate that mercury was also noted in the MB.



Other Issues

One patient sample was observed to have concentrations of total mercury that exceeded the concentration of the highest standard in the calibration curve. The sample result was below the known range of linearity of 242 ug/L total mercury. Results for this sample will be reported as "estimated."

Sample Number	Batch Number	Analysis Date	Reported Value (ug/L total mercury)	Data Qualifier
MN-0752	MN-38	August 12, 2010	234	Estimated result; reported value is over the calibration range but within the known linear range.

The laboratory's SOP has not been revised to indicate that the internal standard is iridium, not rhodium.

Please let me know if you have any questions or concerns with the issues in this report or any other issues regarding the laboratory's role in the study.

SSS

cc: Betsy Edhlund, Research Scientist 2, PHL
Jeff Brenner, Inorganic Chemistry Unit Supervisor, PHL
Paul Swedenborg, Acting Environmental Laboratory Manager, PHL



Memo

DATE: October 28, 2010

TO: Patricia McCann, Principal Investigator and Project Manager
Environmental Health Division

FROM: Suzanne Skorich, Quality Assurance Officer
Public Health Laboratory Division (PHL)

PHONE: (651) 201-5304

SUBJECT: Quality Assurance Review of Mercury Data – Surveillance report #6
Mercury Levels in Blood from Newborns in the Lake Superior Basin
GLNPO Study ID 2007-942

Sixty-eight sets of samples collected during the study have been analyzed for total mercury using ICP-MS. Eight sets of samples have been analyzed since I sent you Surveillance report #5, dated October 6, 2010. For the purposes of this review, issues relative to the quality of analysis of the most recent eight sample sets will be discussed. Issues regarding reporting are not part of the review since sample values have not yet been reported.

The review was conducted using the acceptance criteria established by the MDH standard operating procedure (SOP) entitled “Mercury in Dried Blood Spots by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), Revision D” and the quality assurance project plan (QAPP) entitled “Mercury Levels in Blood from Newborns in the Lake Superior Basin, GLNPO ID 2007-942, Revision 2”.

Deviations from established protocols found during surveillance activities conducted since October 6, 2010 are summarized below. Included is a discussion of corrective actions taken or planned, where necessary.

Because the calibration standards and other quality control check solutions are procedural (prepared and processed in exactly the same manner, and at the same time, as the samples), it is not practical for the analyst to stop an analytical run to take corrective action during the middle of



the run. Therefore, if quality control measures do not meet the established acceptance criteria, the parts of the run between quality control measures, including standards, quality control checks, and samples, must either be re-prepared or the data qualified.

In addition to reporting on the eight sets of samples analyzed since September 17, 2010, outstanding issues from Surveillance reports #1 - #5 will be discussed.

It is my judgment that four sets of data discussed in this report met the measurement quality objectives (MQOs) defined in the SOP and/or QAPP, except where noted. The results from the following sample sets are recommended to be reported.

Sample Set	Analysis Date	Number of samples
MN-52	September 17, 2010	20
MN-53	September 22, 2010	20
MN-55	September 24, 2010	10 (see below)
MN-56	September 30, 2010	20
MN-57	October 1, 2010	20

The samples from the following batches did not meet the measurement quality objectives (MQOs) of the SOP and/or QAPP and are recommended to be reanalyzed (see discussion of individual issues that follow).

Sample Set	Analysis Date	Source of MQO issues
MN-54	September 23, 2010	LCS initial (78%<80%); LCS middle (77%<80%); and No field duplicate precision data because MN-1080 failed to inject during unattended analysis
MN-55	September 24, 2010	The last third of samples and standards failed to inject during unattended analysis
MI-01	October 6, 2010	Calibration not verified (see below)
MI-02	October 7, 2010	Calibration not verified (see below)

Sample Preservation and Sample Storage

There are no deviations from protocol to report on sample preservation and sample storage.



Instrument Calibration, Calibration Verification (CVS), Report Level Verification (RLV)

Instrument data was reviewed to determine how the daily calibration technique of linear regression was applied and whether the acceptance criterion was met. All eight of the samples sets indicated a linear relationship of the standards with correlation coefficients exceeding 0.999 in all cases.

Instrument data was reviewed to determine if the periodic calibration verification steps throughout the analytical run were applied at the correct frequency. Calibration verification is performed at the frequency of once after every 20 samples as described in the SOP.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the calibration verification steps throughout the analytical run. Calibration verification data for batches MI-01 and MI-02 did not pass the acceptance criteria listed in the SOP. Just prior to analyzing these batches, new working standards were prepared from a new lot of stock standard which was purchased because the previous stock calibration standard had expired. The new CVS high level and CVS mid level calibration verification standards recovered at a slightly lower than concentration than those made from the previous stock standard material, which was observed to be closer to the true values than the previous standards were.

The analyst will be conducting analyses of only calibration standards and CVS standards over the course of the next week in order to obtain sufficient data to calculate new limits for the CVS standards. After that time, we will reevaluate whether the calibration verifies for batches MI-01 and MI-02. If so, the data will be recommended to be accepted. Analysis of additional batches will only begin after we are confident that the CVS data meets the criteria for verification.

Finally, instrument data was reviewed to determine whether the check on low level quantitation at the reporting level (RLV) of 2.42 ug/L total mercury met the criterion. All eight of the checks in the batches met the 60-140% recovery criterion as shown below.

Sample Set	Analysis Date	RLV Recovery
MN-52	September 17, 2010	89%
MN-53	September 22, 2010	88%
MN-54	September 23, 2010	84%
MN-55	September 24, 2010	98%
MN-56	September 30, 2010	84%
MN-57	October 1, 2010	84%
MI-01	October 6, 2010	114%
MI-02	October 7, 2010	93%

Holding Time

Holding times have not been established for the method. Since all samples are being held in a freezer, significant degradation of mercury is not expected. There are no deviations from protocol to report on holding times.

QC Accuracy and Precision: Laboratory Control Spikes (LCS), Laboratory Control Spike Duplicates (LCSD), and Quality Control Samples (QCS)

Blank spike (LCS & LCSD) data and certified reference material (QCS) data were reviewed to determine if any recoveries were less 80% or greater than 120%. Except for batch MN-54, the LCS, LCSD, and QCS recoveries were within the 80-120% limits. Precision of the LCS and LCSD in all cases was within the limit of 20% relative percent difference.

Adding to the data provided in Surveillance report #5, the laboratory has thus far collected 199 data points for the QCS and 200 data points for the LCS. Four outliers were identified and removed from the dataset prior to determining the statistically-derived control limits. The following table summarizes the recoveries and control limits that are derived from the data collected.

QC Measure	True Value	N	Mean Recovery	Statistically-derived control limits (mean $\pm$ 3 std dev) calculated from all N	SOP-designated control limits
QCS (SRM 966, bovine blood)	31.4 ug/L	199	94%	76 – 111 %	80 – 120 %
LCS (SRM 966, bovine blood spotted on card)	31.4 ug/L	200	89%	70 – 108%	80 – 120 %

Field Duplicates

All sample sets contained field duplicates; however, since batches MN-54 and MN-55 were not completed due to instrument error, only six of the eight sample sets have associated precision data for field samples. Four of six sets of field duplicates were above the method detection limit (MDL), with three sets showing results between the MDL and the reporting limit (RL). Results between the MDL and the RL are qualified with a “J” signifying estimated concentrations. The remaining set consisted of samples with results above the RL, but having concentrations < 10 times the MDL.

All sets of duplicates met the requirements for precision (results within +/- RL when the calculated concentration is < 10 times the MDL).



Aqueous Blanks

Instrument data was reviewed to determine if the evaluation of low system background using aqueous blanks (AB) throughout the analytical run were applied at the correct frequency. Aqueous blank analysis are performed at the frequency of once after every 20 samples as described in the SOP.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the aqueous blanks throughout the analytical run. Three sets of samples included at least one AB sample over the MDL, but the following AB passed the acceptance criteria. Data is not qualified based on the AB analyses.

Method Blanks

All sample sets contained a method blank (MB) corresponding to each patient sample in the set.

Two instances were noted where a MB was found to be greater than the MDL. In one instance, patient sample MN-1130, the blank indicated a result between the MDL and the RL (marked "J" as estimated concentrations) while the corresponding sample was found to be <RL. Sample results for MN-1130 will be reported as below the RL.

In the other instance the patient sample was substantially greater than the blank value; however, the results of this batch (MI-02) are not being reported at this time.

Other Issues

The laboratory's SOP has been revised to indicate that the internal standard is iridium, not rhodium, and to address other minor changes, errors, or omissions. The SOP is currently under review by the laboratory's management team.

Please let me know if you have any questions or concerns with the issues in this report or any other issues regarding the laboratory's role in the study.

SSS

cc: Betsy Edhlund, Research Scientist 2, PHL
Jeff Brenner, Inorganic Chemistry Unit Supervisor, PHL
Paul Swedenborg, Acting Environmental Laboratory Manager, PHL



Memo

DATE: May 20, 2011

TO: Patricia McCann, Principal Investigator and Project Manager
Environmental Health Division

FROM: Suzanne Skorich, Quality Assurance Officer
Public Health Laboratory Division (PHL)

PHONE: (651) 201-5304

SUBJECT: Quality Assurance Review of Mercury Data – Surveillance report #7
Mercury Levels in Blood from Newborns in the Lake Superior Basin
GLNPO Study ID 2007-942

Eighty-five sets of samples collected during the study have been analyzed for total mercury using ICP-MS. Fourteen sets of samples have been analyzed since I sent you Surveillance report #6, dated October 28, 2010. In addition, one sample set was analyzed on April 20, 2011 using an extended calibration curve in order to verify the concentration of selected samples that were previously analyzed. For the purposes of this review, issues relative to the quality of analysis of the most recent fourteen sample sets will be discussed. Issues regarding reporting are not part of the review since sample values have not yet been reported.

Discussion on the quality of data produced in the sample set identified as “High Results Verification” is not included in this report because the laboratory does not have established quality control limits for the extended calibration range. The calibration verification samples were evaluated against their true values and found to be within 97-104%. The calculated values for the samples in the batch match closely with the results previously obtained. The sample data will be forwarded to you by Betsy Edhlund.

The review was conducted using the acceptance criteria established by the MDH standard operating procedure (SOP) entitled “Mercury in Dried Blood Spots by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), Revision E” and the quality assurance project plan (QAPP) entitled



“Mercury Levels in Blood from Newborns in the Lake Superior Basin, GLNPO ID 2007-942, Revision 2”.

Deviations from established protocols found during surveillance activities conducted since October 28, 2010 are summarized below. Included is a discussion of corrective actions taken or planned, where necessary.

Because the calibration standards and other quality control check solutions are procedural (prepared and processed in exactly the same manner, and at the same time, as the samples), it is not practical for the analyst to stop an analytical run to take corrective action during the middle of the run. Therefore, if quality control measures do not meet the established acceptance criteria, the parts of the run between quality control measures, including standards, quality control checks, and samples, must either be re-prepared or the data qualified.

In addition to reporting on the fourteen sets of samples analyzed since October 28, 2010, outstanding issues from Surveillance reports #1 - #6 will be discussed.

It is my judgment that nine sets of data discussed in this report, along with the samples that were reanalyzed using an expanded calibration curve to verify previous data, met the measurement quality objectives (MQOs) defined in the SOP and/or QAPP, except where noted. The results from the following sample sets are recommended to be reported.

Sample Set	Analysis Date	Number of samples
MI-06	March 8, 2011	20
MI-07	March 9, 2011	20 (see below)
MI-08	March 10, 2011	10 (see below)
MI-09	March 11, 2011	10 (see below)
MI-10	March 15, 2011	10 (see below)
MI-11	March 16, 2011	20 (see below)
MI-03/MN-55	March 17, 2011	20
WI-08B	March 25, 2011	20
MI-04B	April 14, 2011	20
High Results Verification	April 20, 2011	17



The samples from the following batches did not meet the measurement quality objectives (MQOs) of the SOP and/or QAPP and were reanalyzed (see discussion of individual issues that follow).

Sample Set	Analysis Date	Source of MQO issues
MI-04	February 17, 2011	Calibration and recovery issues were found. The batch was reanalyzed on April 14, 2011. All MQOs were met on reanalysis and the results are recommended to be reported.
MI-05	February 18, 2011	Calibration and recovery issues were found. The batch was reanalyzed on April 15, 2011. All MQOs were met on reanalysis, exception for the RLV which recovered over the limit of 140%. The data is recommended to be reported and qualified.

Sample Preservation and Sample Storage

There are no deviations from protocol to report on sample preservation and sample storage.

Instrument Calibration, Calibration Verification (CVS), Report Level Verification (RLV)

Instrument data was reviewed to determine how the daily calibration technique of linear regression was applied and whether the acceptance criterion was met. All but one of the fifteen samples sets indicated a linear relationship of the standards with correlation coefficients exceeding 0.999 in all cases. The exception was the first batch MI-04 which was reanalyzed as sample set MI-04B, and the data from MI-04B was found to be acceptable.

Instrument data was reviewed to determine if the periodic calibration verification steps throughout the analytical run were applied at the correct frequency. Calibration verification is performed at the frequency of once after every 20 samples as described in the SOP.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the calibration verification steps throughout the analytical run. Calibration verification data for the following batches did not pass the acceptance criteria listed in the SOP because the final CVS-high standard and following aqueous blanks failed to inject during unattended operation of the instrument.

Sample Set	Analysis Date	Calibration verification issues
MI-08	March 10, 2011	Final CVS-high and final blanks did not inject
MI-09	March 11, 2011	Final blanks did not inject
MI-10	March 15, 2011	Final CVS-high and final blanks did not inject
MN-43B	March 24, 2011	Final CVS-high and final blanks did not inject; RLV recovery high
MI-02/MN-19/MN-37	March 29, 2011	Initial CVS-high outside limits; final CVS-high and final blanks did not inject; RLV recovery high

I recommend that the samples in the following samples sets identified with the calibration issues listed above and resulting from the instrument malfunction are reported and qualified using the “PC” qualifier to indicate that the calibration was partially verified . The justification for reporting the data is that most results were within the verified portion of the curve. In addition, the last two sample sets in the list shall be reported with the “RV2” qualifier to indicate that the RLV recovery was over the acceptance limit (see discussion on RLV below for justification).

Sample Set	Number of samples to qualify	Justification(s) for qualification
MI-08	10	All ten patient samples and associated blanks are below the RL with no values between the MDL and the RL (J flag qualifier).
MI-09	10	Nine of ten patient samples and associated blanks are below the RL with five values between the MDL and the RL (J flag qualifier); one sample exceeded the RL.
MI-10	10	All ten patient samples and associated blanks are below the RL with three values between the MDL and the RL (J flag qualifier).
MN-43B	20	Fourteen of nineteen patient samples and associated blanks

		are below the RL with nine values between the MDL and the RL (J flag qualifier); five samples exceeded the RL.
MI-02/MN-19/MN-37	20	Thirteen of twenty patient samples and associated blanks are below the RL with nine values between the MDL and the RL (J flag qualifier); six samples exceeded the RL.

Finally, instrument data was reviewed to determine whether the check on low level quantitation at the reporting level (RLV) of 2.42 ug/L total mercury met the criterion. Nine of the fourteen checks in the batches met the 60-140% recovery criterion as shown below. The remaining five checks were recovered greater than the upper limit of 140%.

Sample Set	Analysis Date	RLV Recovery
MI-06	March 8, 2011	79%
MI-07	March 9, 2011	133%
MI-08	March 10, 2011	137%
MI-09	March 11, 2011	127%
MI-10	March 15, 2011	90%
MI-11	March 16, 2011	112%
MI-03/MN-55	March 17, 2011	116%
MN-51B	March 18, 2011	145%
MN-43B	March 24, 2011	146%
WI-08B	March 25, 2011	126%
MI-02/MN-19/MN-37	March 29, 2011	160%
WI-03/MN-44B	March 30, 2011	162%
MI-04B	April 14, 2011	139%
MI-05B	April 15, 2011	143%

Since the failing RLV in all instances indicates a high bias, I contend that the recoveries of the RLV do not negatively impact the overall quality of the data in these samples sets. The number of samples that, in my judgment, may have been biased is summarized in the table below.



Additionally, all of the sample sets for which the RLV was over the upper limit contain at least some samples for which there are insufficient dried blood spots remaining to repeat the test. Therefore, I recommend that results for samples in the following sets be qualified to indicate that the RLV did not meet the MQOs of the project.

Sample Set	Analysis Date	Number of samples >RL / total number of samples in set
MN-51B	March 18, 2011	1 / 20
MN-43B	March 24, 2011	5 / 20
MI-02/MN-19/MN-37	March 29, 2011	7 / 20
WI-03/MN-44B	March 30, 2011	2 / 20
MI-05B	April 15, 2011	1 / 20

Holding Time

Holding times have not been established for the method. Since all samples are being held in a freezer, significant degradation of mercury is not expected. There are no deviations from protocol to report on holding times.

QC Accuracy and Precision: Laboratory Control Spikes (LCS), Laboratory Control Spike Duplicates (LCSD), and Quality Control Samples (QCS)

Blank spike (LCS & LCSD) data and certified reference material (QCS) data were reviewed to determine if any recoveries were less 80% or greater than 120%. Except for batch MN-51B, the LCS, LCSD, and QCS recoveries were within the 80-120% limits. Except for batch MI-11, precision of the LCS and LCSD in all cases was within the limit of 20% relative percent difference.

The three LCS samples in batch MN-51B recovered at 75%, 79%, and 76%. These values are within the statistically-derived control limits of 70-108%. I recommend the results of the batch be reported and the results qualified for accuracy.

The RPD for the first set of LCS in batch MI-11 was calculated at 27%. I recommend the results of the batch be reported and the results qualified for precision.

Adding to the data provided in Surveillance report #6, the laboratory has thus far collected 244 data points for the QCS and 245 data points for the LCS. Four outliers were identified and removed from the dataset prior to determining the statistically-derived control limits. The following table summarizes the recoveries and control limits that are derived from the data collected.

QC Measure	True Value	N	Mean Recovery	Statistically-derived control limits (mean $\pm$ 3 std dev) calculated from all N	SOP-designated control limits
QCS (SRM 966, bovine blood)	31.4 ug/L	244	94%	77 – 111 %	80 – 120 %
LCS (SRM 966, bovine blood spotted on card)	31.4 ug/L	245	89%	70 – 108%	80 – 120 %

Field Duplicates

All sample sets contained field duplicates. Six of fourteen sets of field duplicates were above the method detection limit (MDL), with all six sets showing results between the MDL and the reporting limit (RL). Results between the MDL and the RL are qualified with a “J” signifying estimated concentrations. None of the samples sets contained field duplicate results above the RL.

All sets of duplicates met the requirements for precision (results within +/- RL when the calculated concentration is < 10 times the MDL).

Aqueous Blanks

Instrument data was reviewed to determine if the evaluation of low system background using aqueous blanks (AB) throughout the analytical run were applied at the correct frequency. Aqueous blank analysis are performed at the frequency of once after every 20 samples as described in the SOP. As noted in the calibration verification discussion, five sample sets were not completed during unattended analysis, leaving the final ABs unanalyzed.

Instrument data was reviewed to determine whether the acceptance criteria were met for all of the aqueous blanks throughout the analytical run. Eight sets of samples included at least one AB sample over the MDL, but the following AB passed the acceptance criteria. Data is not qualified based on the AB analyses.

Method Blanks

All sample sets contained a method blank (MB) corresponding to each patient sample in the set.

In one instance, patient sample MN-0368 analyzed on March 29, 2011, the blank indicated a result between the MDL and the RL (marked “J” as estimated concentrations) while the corresponding



sample was found to be <RL. Sample results for MN-0368 will be reported as below the RL and qualified to indicate that the sample blank was found to be higher than the authentic sample.

In two other instances, the MB did not inject; however, the corresponding patient sample was found to be <RL. The samples are MI-0117 analyzed on March 9, 2011 and MN-1006 analyzed on March 18, 2011. Sample results for MI-0117 and MN-1006 will be reported as below the RL.

Other Issues

The laboratory's revised SOP (Revision E) has been accepted by the Public Health Laboratory's management.

Five samples have not been successfully analyzed in the course of the study. The unanalyzed samples are: MN-0859, MN-0747, MN-0760 Dup, MN-1080, and MN-1080 Dup. Due to the large percentage of samples that have been successfully analyzed, I recommend that the laboratory not pursue further analysis of these missed samples.

If you are agreeable to concluding the study upon receipt of the data for the sample sets reviewed in this report, I will forward a final quality control assessment for the project by the end of May 2011.

Please let me know if you have any questions or concerns with the issues in this report or any other issues regarding the laboratory's role in the study.

SSS

cc: Betsy Edhlund, Research Scientist 2, PHL
Jeff Brenner, Inorganic Chemistry Unit Supervisor, PHL
Paul Moyer, Environmental Laboratory Manager, PHL

Memo

DATE: June 30, 2011

TO: Patricia McCann, Principal Investigator and Project Manager
Environmental Health Division

FROM: Suzanne Skorich, Quality Assurance Officer
Public Health Laboratory Division (PHL)

PHONE: (651) 201-5304

SUBJECT: Quality Assurance Review of Mercury Data – Final Report
Mercury Levels in Blood from Newborns in the Lake Superior Basin
GLNPO Study ID 2007-942

The Environmental Laboratory has concluded the analysis of 1496 samples in the study for total mercury using ICP-MS. For the purposes of this review, issues relative to the quality of analysis of the entire study conducted from 2009 through 2011 will be discussed.

The review was conducted using the quality assurance project plan (QAPP) entitled “Mercury Levels in Blood from Newborns in the Lake Superior Basin, GLNPO ID 2007-942, Revision 2”.

Section A. Project Management

Sections A1 through A6 are not included in the review since these sections are descriptive of the project and not related to the quality of the data. A discussion on Sections A7 through A9 follows.

A7. Data Quality Objectives for Measurement Data

The sensitivity of the method used in the course of the study was sufficient to meet the data quality objective of 5.8 ug/L total mercury in blood.

Measures taken during the course of the study to identify potential contamination from handling samples and cards in varying hospital and laboratory environments, as well as transport and storage of the samples indicated few areas of concern for contamination. These measures are discussed in greater detail in the section entitled “Method Blank (MB)” below.

Supplies and consumables were tested for mercury throughout the course of the project. Supplies and consumables all tested negative for total mercury prior to placing these items into use.

Table 1. of the QAPP defined measurement quality objectives (MQOs) for the study. Below is an assessment of each item with discussion on how issues encountered throughout the study may have impacted the quality of the results.

Reporting Units

The reporting unit for the study was ug/L total mercury in blood. There were no issues with the reporting unit for sample results.

All samples were analyzed using a calibration curve that covered the range of concentrations of total mercury found in patient samples.

The range of results found in patient samples was 0.700 ug/L total mercury to 225 ug/L total mercury.

The range of results found in the blank filter card associated with patient samples was 0.700 ug/L total mercury to 3.93 ug/L total mercury.

Instrument Detection Limit (IDL)

The IDL was determined as 0.001 ug/L total mercury on 8/21/2009.

A daily performance check along with internal standard intensities were monitored throughout the study to assure that the instrument was operating with sufficient sensitivity to quantitate patient samples and blanks as low as the reporting limit and to provide an estimate on results between the method detection limit and the reporting limit.



Preventive maintenance, including cleaning of the sample introduction system, the nebulizer, injector, and skimmer and sampler cones, was performed to assure that the analytical system was routinely optimized.

Method Detection Limit (MDL)

The method detection limit (MDL) is defined as the minimum concentration of a target analyte that can be measured and reported with 99% confidence that the concentration is greater than zero. For the procedure in use during the study, the steps to determine the MDL are found in 40 CFR Part 136, Appendix B.

The MDL was determined prior to analyzing patient samples. A filter card was spotted with human blood spiked with a mixture of methyl mercury and inorganic mercury at a concentration of 2.13 ug/L and allowed to air dry overnight.

Ten replicate samples consisting of two punches of blood spots for each replicate sample were carried through the entire analytical process on July 21-22, 2009. The MDL was calculated from the precision of the study using the Student's t-test value for nine degrees of freedom multiplied by the standard deviation of the ten replicate total mercury values. The MDL was determined as 0.79 ug/L.

At the conclusion of the study, we determined that the concentrations of the purchased stock methyl mercury standards used throughout the study were incorrectly assigned by the laboratory. This error was noted when a new standard was obtained from a different vendor and found to differ from the initial vendor's concentration of methyl mercury by a value that was approximately 23% higher than attested to by the manufacturer of the initial vendor's standard. After discussions with both vendors, and verification of both standard concentrations by another laboratory, we concluded that the error would affect the quality of the results of the study, and all data must be reprocessed.

Intermediate and working standards prepared in the laboratory for the study consisted of a 50:50 v/v mixture of the purchased methyl mercury standard and a purchased inorganic mercury standard. Therefore, the error that may have been present in the standards used in the MDL study due to the misrepresented methyl mercury standard is estimated to be up to 11%. Six different lots of methyl mercury were used to quantify results throughout the study, including one lot which was used to determine the MDL.

The manufacturer of the standard provided us with data, demonstrated at the time the standard was certified, indicating the correct concentration of mercury (as total mercury) in the methyl mercury standards used throughout the study. All data, including the MDL



study, have been reprocessed using the corrected concentration of methyl mercury in the stock calibration standards.

The reprocessed data for the MDL study were used to recalculate the MDL. The MDL should have been reported as 0.70 ug/L total mercury in blood.

Patient samples were reported as estimated values if their observed total mercury concentration was between the MDL of 0.70 ug/L and the reporting limit (RL) of 2.13 ug/L total mercury.

No other issues were encountered with the MDL study.

Reporting Level Verification (RLV)

The reporting level (RL) is defined as the lowest concentration of a target analyte that can be reliably measured within specified limits of precision and accuracy, during routine laboratory operating conditions. The report level is verified with each analytical run to demonstrate that the reporting level is valid within the analytical run.

For the project, defined limits for the reporting level verification (RLV) standard were not agreed on in the QAPP; however, the laboratory has an established guideline that the percent recovery of the RLV standard must fall within $\pm 40\%$ of the true value.

The RLV was prepared by spotting a filter card with human blood spiked using a 50:50 v/v mixture of methyl mercury and inorganic mercury equivalent to a concentration of 2.42 ug/L when two card punches were analyzed together. The spotted cards were allowed to air dry overnight prior to storage for use in the study.

The concentration error found in the methyl mercury standards described above affected the values initially reported to you. Two different lots of methyl mercury were used to produce the RLV standards for the entire study. One lot was used from December 23, 2009 to April 30, 2011 and was made from the same lot of standard as was used to produce the calibration standards on December 23, 2009. Using the corrected concentration for methyl mercury provided by the vendor, the concentration in the RLV standard for this lot is equivalent to a concentration of 2.13 ug/L total mercury when two card punches were analyzed together.

The other lot was used from March 1, 2011 to the conclusion of the study and was from a different lot than that used for the calibration standards. Using the corrected concentration for methyl mercury provided by the vendor, the concentration in the RLV standard for this



lot is equivalent to a concentration of 2.21 ug/L total mercury when two card punches were analyzed together.

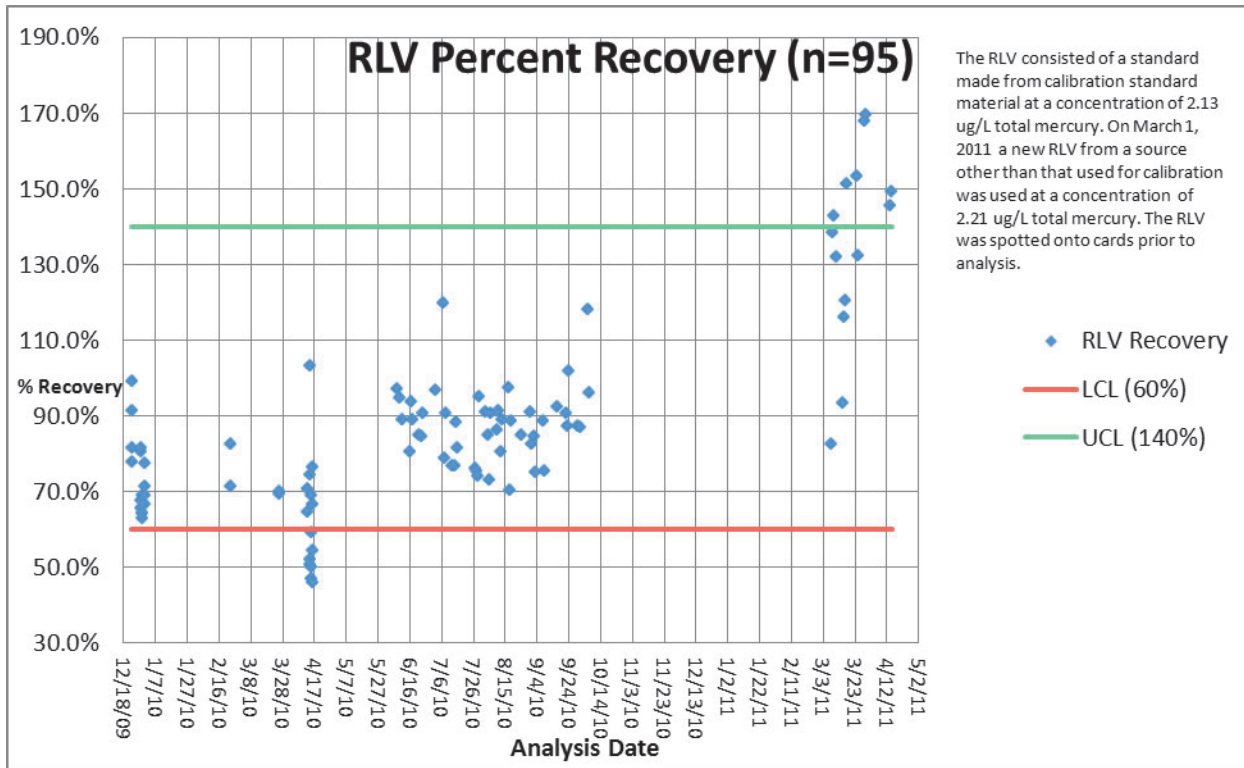
All data, including the RLV standards, have been reprocessed using the corrected concentration of methyl mercury in the stock calibration standards.

Initially, and through April 2010, the RLV standard was analyzed twice per analytical run, although the QAPP requirements for only one RLV check per analytical run. After April 2010, the RLV was analyzed once per analytical run. Ninety-five RLV determinations were analyzed throughout the study.

Patient samples were reanalyzed in another batch or qualified whenever the RLV standard did not fall within the lower control limit (LCL) of 60% recovery and the upper control limit (UCL) of 140% recovery.

Of the 1496 patient samples analyzed, 98 samples (7% of all samples analyzed) were qualified due to a high recovery of the RLV standard and 19 samples (1% of all samples analyzed) were qualified due to a low recovery of the RLV standard.

The chart below indicates the recovery of the RLV standard throughout the course of the study.



The exact cause of the shift in RLV recovery after March 1, 2011 is not understood. The standard material used to produce the second batch of RLV standards, when analyzed without spotting the sample onto the card, also produced a slightly high bias of about 12%.

Calibration Verification (CVS)

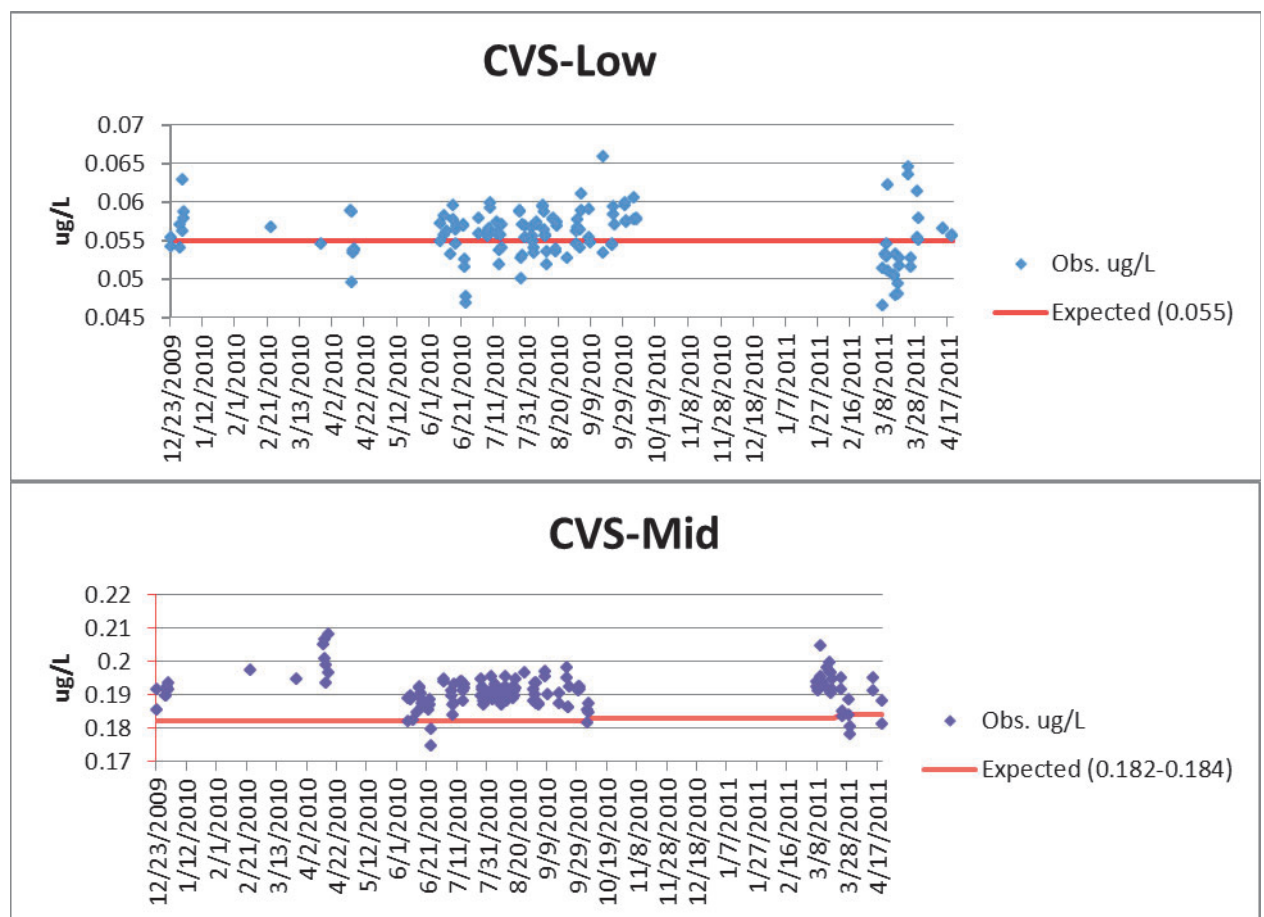
The instrument was calibrated every day of use with an aqueous blank and five standards of increasing concentration from the reporting level to approximately 48 ug/L total mercury. The standards were prepared in water, acid, and diluent using a 50:50 v/v mixture of methyl mercury and inorganic mercury.

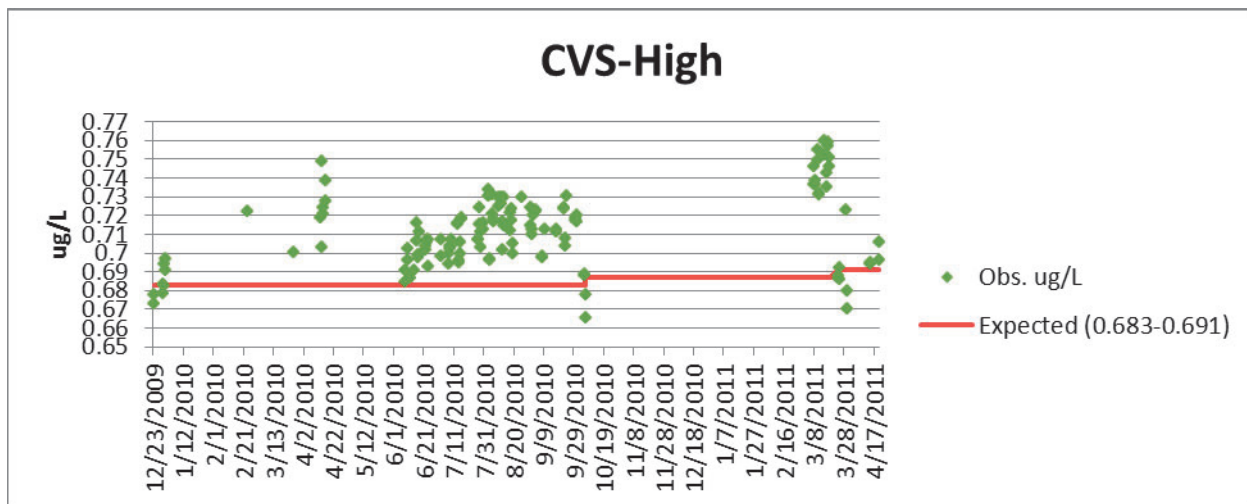
The concentrations of the calibration standards were incorrectly assigned at the time of analysis, but were adjusted prior to reprocessing all sample data to reflect the true concentrations of the mercury standard lots used during the study. The corrected values of the five calibration standards differed slightly in total mercury concentration with each change in lot of methyl mercury standard. Therefore, the calibration range was not consistent for the entire study, but varied slightly from lot to lot.

Calibration verification standards (CVS) at three levels (low, medium, and high) were prepared from the same lots of standards as those used to prepare the calibration standards. Patient samples were bracketed by CVS pairs and the average of the pairs were used to evaluate the on-going acceptability of the calibration.

The concentration error found in the methyl mercury standards described above affected the CVS-low, CVS-medium, and CVS-high standards as well. All CVS data was reprocessed, taking into account the changes in concentrations from lot to lot.

The following charts show the observed values for the CVS-low, CVS-medium, and CVS-high standards for the entire study. Each data point represents the average of the CVS pairs for the analytical run.





The CVS-medium and CVS-high standards showed a bias of up to 10% for much of the study.

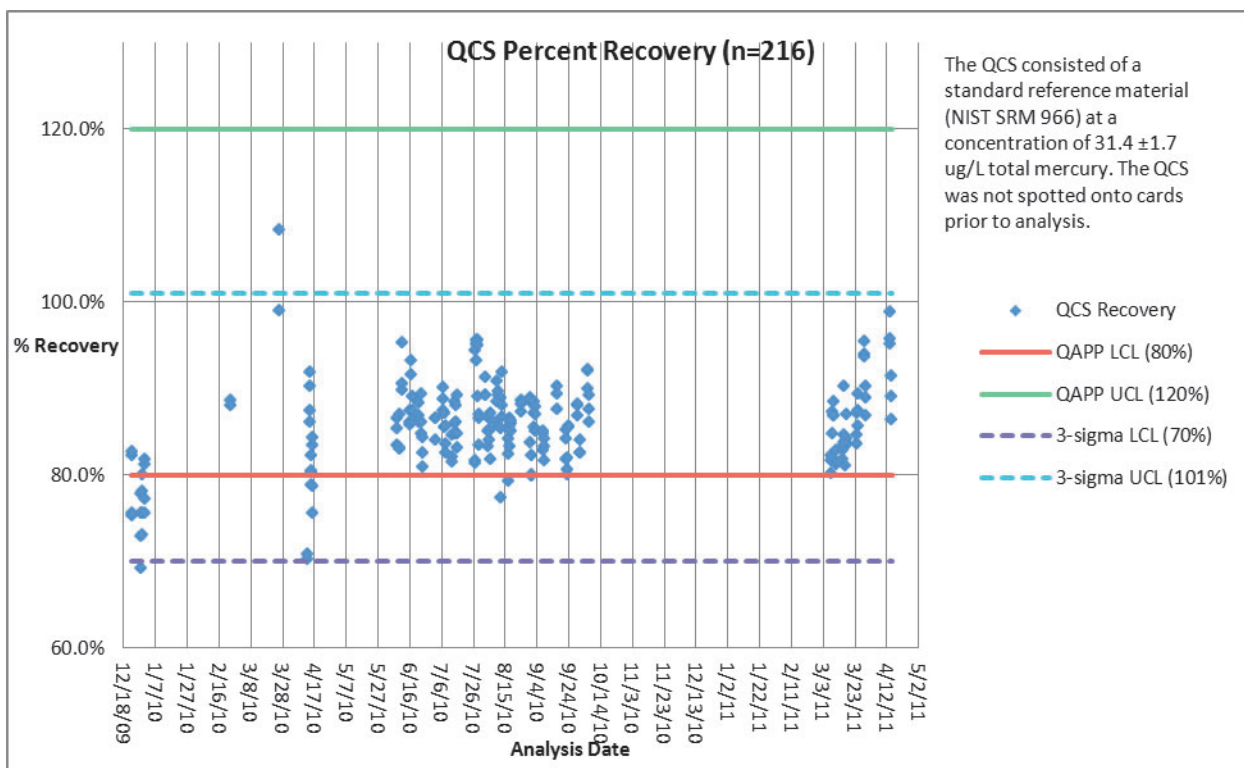
We have not been able to determine the exact cause of this bias. We suspect that some of the bias could be attributed to the mechanical pipet used to prepare the CVS-medium and the CVS-high verification standards along with the highest calibration standard. All other standards were prepared using a 1000 uL adjustable pipet. The pipet used to make the CVS standards in question is adjustable up to 5000 uL and is calibrated by an outside calibration company every six months. Calibration data for the 5000 uL pipet was reviewed, and the data shows that the pipet met the accuracy of 2.5% and the precision criteria of 1.0%. However, there is an indication that for three of the four calibration events during the study a possible 1% high accuracy bias may have been noted. This is not sufficient to ascribe the high bias in the CVS standards, but it may have been a contributing factor. A similar investigation of the 1000 uL adjustable pipet has not been performed.

Quality Control Sample (QCS)

The QCS was used throughout the analytical run to demonstrate that mercury could be extracted from blood. The material used in the QCS is a NIST standard reference material (SRM) of bovine blood at a concentration of 31.4 ± 1.7 ug/L total mercury.

At the outset of the project, the laboratory did not have experience with how well mercury would be extracted from either whole blood or blood that had been spotted onto cards and then dried and carried through the entire analytical procedure. The acceptance criteria published in the QAPP of 80-120% recovery were selected based on acceptance criteria recommended in EPA methods for metals in water matrices.

The following graph demonstrates the extraction efficiency for total mercury in the QCS for the entire study. The average recovery of the QCS was 86%. Using all of the data collected the recovery of QCS limits are calculated, using the average $\pm$ 3-sigma, as 70-101%.



Patient samples were reanalyzed in another batch or qualified whenever the QCS standard did not fall within the lower control limit (LCL) of 80% recovery and the upper control limit (UCL) of 120% recovery. If insufficient sample remained to reanalyze the samples, the results were qualified.

Of the 1496 patient samples analyzed, 248 samples (17% of all samples analyzed) were qualified due to a recovery of the QCS outside the 80-120% acceptance limits.

Laboratory Control Sample (LCS)

The LCS was used throughout the analytical run to demonstrate that mercury could be extracted from dried blood spots. The material used in the LCS is the same NIST standard reference material (SRM) of bovine blood used for the QCS at a concentration of $31.4 \pm$



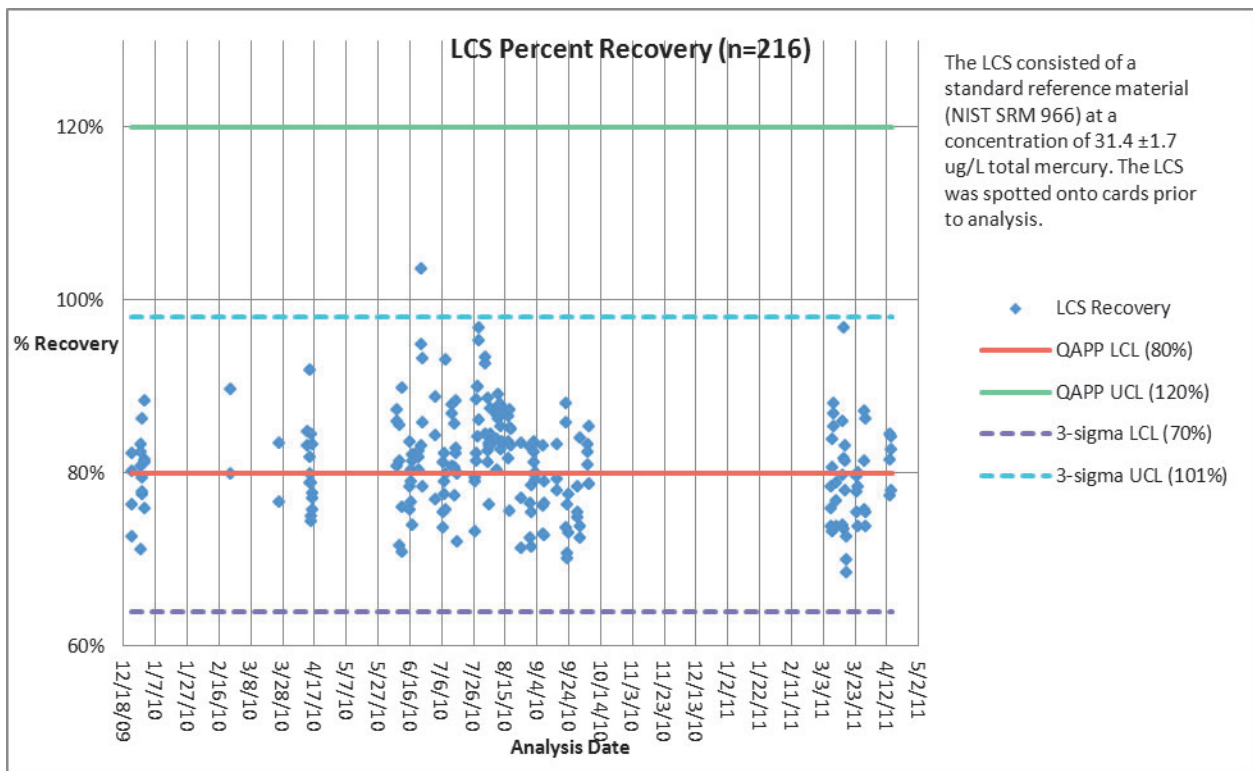
1.7 ug/L total mercury. The difference is that the LCS was spotted onto cards, allowed to air dry, and hand punched into the well plate for analysis. Unlike the QCS, each LCS was analyzed in duplicate, using a separate set of 2 punches into another well, to obtain precision data (see discussion below).

LCS Accuracy

At the outset of the project, the laboratory did not have experience with how well mercury would be extracted from either whole blood or blood that had been spotted onto cards and then dried and carried through the entire analytical procedure. The acceptance criteria published in the QAPP of 80-120% recovery were selected based on EPA methods for metals in water matrices.

Of the 1496 patient samples analyzed, 907 samples (60% of all samples analyzed) were qualified due to a recovery of the LCS outside the 80-120% acceptance limits.

The following graph demonstrates the extraction efficiency for total mercury in the LCS for the entire study. The average recovery of the LCS was 81%. Using all of the data collected, the recovery of LCS limits are calculated, using the average $\pm$ 3-sigma, as 64-98%.

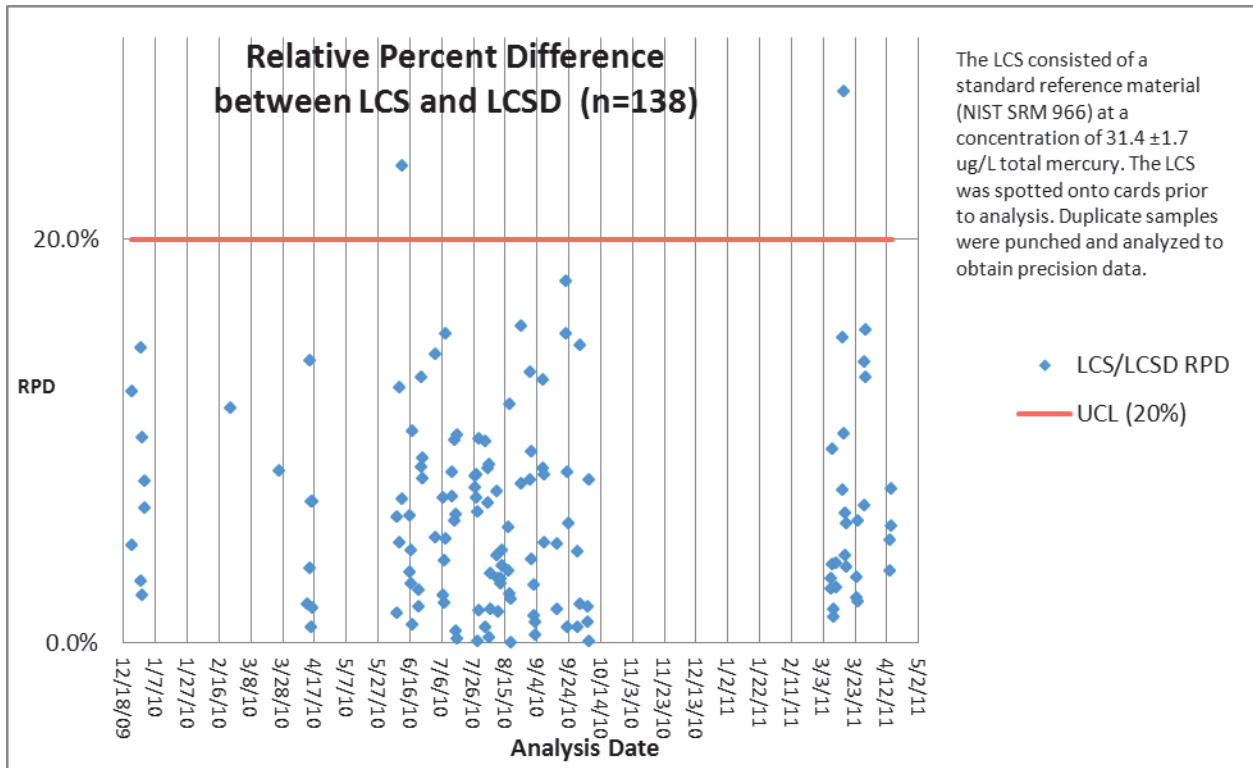


LCS Precision

Duplicate LCS samples were included in each batch of patient samples analyzed in order to evaluate the variability of the analytical procedure. The acceptance criteria for LCS/LCSD precision was 20%.

Two instance were noted where the precision for the LCS/LCSD pair did not meet the criteria. Of the 1496 patient samples analyzed, 40 samples (3% of all samples analyzed) were qualified due to RSD of the LCS greater than the 20% acceptance limits.

The following graph demonstrates the precision for total mercury in the LCS for the entire study. The average RPD of the LCS/LCSD pairs was 6.4%.



Reference Sample (RS)

Reference samples (RS) were used to assess the transport and storage of patient samples from three separate state's newborn screening programs.

Reference samples were made from the same standard reference material used for the LCS; however, the reference material, after being spotted onto cards in the laboratory, were submitted in bulk to each of the newborn screen programs from the three states. Newborn screening personnel used the cards to insert RS samples among the patient samples as if they were authentic patient samples.

The knowledge of which samples constituted the RS is not known to the laboratory, therefore, I am unable to assess whether the frequency of submission was adequate or whether the 80-120% acceptance limit was consistently reached.

Internal Standard (IS)

Internal standards were added to each standard, blank, patient sample, and method blank. The response for IS was generally very consistent over the course of an analytical run. The



only data that was impacted by an IS that did not meet the criteria of $\pm 50\%$ of the average response of the IS in the five calibration standards were those samples or standards that failed to inject due to instrument malfunction.

Patient samples were reanalyzed in another batch whenever the IS did not fall within the limit. A few patient samples did not have sufficient sample remaining and those samples were dropped from the study.

Aqueous Blank (AB)

Aqueous blanks (AB) were used to assure that reagents were not contaminated with mercury or that carryover of higher levels of mercury was not occurring between the highest calibration standard or LCS and patient samples.

Of the 78 batches of samples with reportable results, only one batch (MN-43B analyzed on March 24, 2011) had an AB preceding a patient sample that exceeded the MDL of 0.7 ug/L total mercury. The sample immediately after the high AB was observed below the MDL, therefore, no impact on data quality can be attributed to the AB in question.

Method Blank (MB)

For each patient sample punched from their blood specimen spotted onto a filter card, a blank area of the card was also punched to use as a method blank. The method blanks were processed in a manner identical to the associated patient samples.

Of the 1496 method blanks analyzed, no method blanks were observed with mercury values over the reporting limit (RL). Sixteen method blanks (1% of blanks analyzed) were estimated to be between the MDL and the RL. Of the sixteen method blanks with low levels of total mercury, only seven (less than 1% of samples analyzed) were observed with mercury values in the blank card punches while those of the accompanying patient sample were free of mercury.

Field Duplicate (FD)

Field duplicates (FD) consisted of spots punched in duplicate of patient samples. The well plates arrived with the duplicate samples in place.

Eighty FD pairs were analyzed in the course of the project. Seven of the FD pairs were observed over the reporting level (RL). One pair of FD (MN-0620) failed the precision limit of 20% relative standard deviation (RSD) with its RSD calculated at 21%. The patient sample result was qualified. All other FD pairs met the criteria for precision.



The 73 FD pairs that were observed below the RL but over the MDL met the criteria for precision, meaning that the results were within $\pm$ RL.

Laboratory Duplicate (LD)

At the time that the QAPP was written, the laboratory expected to receive eight dried blood spots for each patient and sixteen for the FD pairs. Thus, sufficient sample would have been available to perform the laboratory duplicates (LD) described in the QAPP.

However, as the newborn screening laboratory personnel started to punch samples for the study it became evident that there was insufficient sample to provide the number of spots initially agreed on. At about the same time, the laboratory switched to using 96 well plates allowing the Research Scientist to scale down the extraction volumes and use fewer blood spots for analysis. This allowed patients for which slightly smaller amounts of blood spots remained on the cards to be included in the study. Hence, the laboratory was provided only four dried blood spots for each patient and eight for the FD pairs, which was only enough spots for two analytical runs. The LD procedure was modified from the outset of sample analysis.

To replace patient samples as the LD, the laboratory substituted dried blood spots of past PT samples at two separate concentrations, 5.36 ug/L and 15.9 ug/L. The PT samples were spotted onto cards, allowed to air dry, and hand punched in duplicate into the well plates. One of PT samples was analyzed at the beginning of the analytical run and the other was analyzed at the end of the run.

The table below summarizes the results.

PT Sample ID	Assigned Value (ug/L total mercury)	Number analyzed	Average Recovery	Average RSD
0948	15.9	92	92%	6.8%
0949	5.36	93	84%	8.8%

There were no issues impacting data quality with the LD-substituted PT samples.

A8. Special Training Requirements/Certification

All laboratory personnel, including those involved with sampling, performed their functions for the project as expected. There were no training issues that could be identified which may have impacted the quality of the results.

The Research Scientist responsible for the mercury analysis participated in proficiency testing (PT) programs through the Centers for Disease Control and Prevention (CDC) for which she analyzed challenge samples for mercury in human blood (whole blood) throughout the time frame of the mercury in dried blood spot project.

There were no issues stemming from the analysis of the PT samples as noted in the following table.

PT Study	Study Closing Date	Number of Challenge Samples	Evaluated as Acceptable
2009-03	9/23/2009	10	100%
2010-01	2/19/2010	5	100%
2010-02	6/11/2010	5	100%
2010-POP (unannounced PT)	9/15/2010	10	100%
2010-03	10/22/2010	5	100%
2011-01	2/11/2011	5	100%

A9. Documentation and Record

All records required in the QAPP were maintained, including laboratory notebooks, instrument (raw) data files, and final processed data.

Reports were provided to you as the samples were analyzed. However, due to the error in standard concentration of methyl mercury, all reported values were amended and reported in one report.

A final data report for all samples consisting of the amended values was provided to you by the Research Scientist on June 27, 2011. The report contained all of the data required for patient samples and included a tabulated summary of quality control determinations by batch.

Project data will be maintained in the laboratory for one year and for the additional nine years in an off-site record storage location. Electronic data will be maintained in the laboratory and will be retrievable for the full 10-year retention period.

Section B. Data Generation and Acquisition

Sections B1 through B3 are not included in the review since these sections were out of the control of laboratory personnel. A discussion on Sections B4 through B10 follows.

B4. Analytical Methods Requirement

Several revisions of the laboratory's analytical method were produced during the course of the project. The table below summarizes the substance of the revisions.

Revision	Revision Date	Revision History
A	February 28, 2008	Initial release
B	June 28, 2008	Fixed typos, added more procedural details
C	November 26, 2008	New autosampler
D	June 11, 2009	Changed number of punches, volumes, and move to 96-well plates
E	February 7, 2011	Corrected internal standard and wording

Although there were several changes to the documented standard operating procedure, all samples were analyzed using the same equipment and source of supplies throughout the project. Therefore, it is my judgment that the revisions to the analytical method did not affect the quality of the reported results.

B5. Quality Control Requirement

The previous section describes in some detail the specific challenges encountered in the study. The fact that the QCS and LCS determinations did not meet the 80-120% acceptance limits is a reflection more on the selection of acceptance limits that are based on aqueous matrix analyses, which are considerably less challenging than a blood-based matrix, than on the ability of the method and the laboratory to extract and quantify total mercury in dried blood spots.

Overall, in my opinion, the measurement quality objectives of the project were met and the data fits the purpose of the study as defined in the QAPP.

Sample Collection Quality Control

At the time that the QAPP was written, the expectation was that samples would be punched by hand. Due to the demanding effort for the newborn screening personnel, hand punching



the samples was abandoned after about 100 samples and replaced with automated punching. There was no significant difference between the two sampling methods.

Filter blank cards do not appear to contribute appreciable levels of mercury to the sample results.

Precision data of field duplicates (FD) support the assumption that there was good distribution of blood specimens on the card and the punching devices produced consistent punch spot size.

Pre-populated chain-of-custody forms appeared to enhance the accuracy of sample collection, since no discrepancy was found between sample documentation and chain-of-custody forms upon arrival at the laboratory.

Analytical Quality Control

Precision: Field duplicates indicated good precision, with only one sample exceeding the limit of $\pm 20\%$ RSD.

Accuracy: The percent recovery of some of the QCS and more of the LCS samples were not within the established limits of 80-120% recovery.

During the course of the study, the Research Scientist, the Inorganic Chemistry Unit Supervisor and myself investigated many factors that may have improved on the recovery of the QCS and LCS samples. The conclusion we have drawn is that the extraction efficiency of mercury from both whole blood and dried blood spots is less than we initially predicted.

Background Assessment: The low system background determinations (AB and MB samples) indicate little problem with background for mercury in the analytical procedure or the blank filter paper above the MDL, much less the RL.

Sensitivity: The IDL, MDL, RLV, and IS were all used to assess the sensitivity of the instrument. Preventive maintenance was used to assure that the ICP-MS was optimized. Careful attention by the Research Scientist to cleaning the sample introduction apparatus allowed adequate sensitivity to be maintained for the project.



Completeness: The following table summarizes the completeness

Element of Valid Sample	# Valid / # Received	% Valid Samples in Study
Arrived to lab intact	1500 / 1500	100%
Had sufficient quantity	1496 / 1500	98%
Chain-of-custody form complete	1500 / 1500	100%
All QC acceptance criteria met, including 80-120% for QCS & LCS	500/ 1500	33%

Clearly the laboratory did not meet the goal of 96% completeness, due to the inability to meet the 80-120% recovery of the QCS and LCS samples.

As stated earlier, I believe that, had the laboratory had experience with the extraction efficiency of mercury using the analytical method prior to providing the acceptance criteria contained in the QAPP, we would not have provided such stringent limits.

Representativeness: There is a lack of data to assess the representativeness of the samples; however, the precision data of the field duplicates support that the samples were homogeneous within the spots tested.

Comparability: The laboratory's data is not being compared against another laboratory's data.

B6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements

Instruments and equipment were inspected and maintained as required in the QAPP.

There were no specific issues that may have affected the quality of the data with the test equipment encountered, other than those described in detail above.

B7. Instrument/Equipment Calibration and Frequency

The calibration routine described was followed; however the second-source calibration verification was conducted with a different lot of standard from the same vendor. This practice was used because it was not possible to find a second-source and traceable standard from another vendor until just prior to the conclusion of the study.

Due to a misunderstanding of the vendor's certificate of analysis on the laboratory's part, the methyl mercury standard was erroneously ascribed to be 1000 ± 5 mg/uL as methyl mercury,



rather than as methyl mercuric (II) chloride. Our mistake stemmed from the certificate not clearly stating the form of mercury certified, as well as the fact that the standard was in a liquid form, not a salt form.

This error impacted the quality of the results; however, the data was recalculated and we are confident that the results are valid.

B8. Inspection/Acceptance of Supplies and Consumables

Supplies and consumables were tested prior to use and were found to be suitable. No issues were encountered that may have impacted the quality of the results.

B9. Non-direct Measurements

There are no data required from non-measurement sources for the project.

B10. Data Management

During the course of the study, the laboratory changed its LIMS data system. To date, the data has not been transferred from the old LIMS to the new LIMS, but the laboratory is discussing on how to proceed with the transfer.

There were no other data management issues during the study.



Conclusion

In my opinion and to my best ability, I confirm that the data for this study were accumulated, transferred, reduced, calculated, summarized, and reported correctly.

I recommend that all the 1496 patient sample results be included in the data decision-making process, despite a large percentage of the QCS and LCS data failing to meet the 80-120% acceptance limits. The validity of accepting the qualified data is based on the assumption that the extraction efficiency of patient samples is similar to that of the LCS.

Please let me know if you have any questions or concerns with the issues in this report or any other issues regarding the laboratory's role in the study.

SSS

cc: Betsy Edhlund, Research Scientist 2, PHL
Jeff Brenner, Inorganic Chemistry Unit Supervisor, PHL
Paul Moyer, Environmental Laboratory Manager, PHL

Memo

DATE: July 28, 2011

TO: Patricia McCann, Principal Investigator and Project Manager
Environmental Health Division

FROM: Suzanne Skorich, Quality Assurance Officer
Public Health Laboratory Division (PHL)

PHONE: (651) 201-5304

SUBJECT: Quality Assurance Review of Mercury Data – Supplement I to Final Report
Mercury Levels in Blood from Newborns in the Lake Superior Basin
GLNPO Study ID 2007-942

On June 30, 2011, you asked me to expand on the information I sent to you in my memo of June 30, 2011 designated “Quality Assurance Review of Mercury Data –Final Report Mercury Levels in Blood from Newborns in the Lake Superior Basin, GLNPO Study ID 2007-942.”

Additional information is provided on the following topics:

1. the linearity of the instrument for the range of sample concentrations observed;
2. charts of the observed values of calibration standards similar to those provided for CVS data; and
3. a discussion on weaknesses of the method and/or areas of the analytical aspects of the study that could be adjusted in order to improve future use of dried blood spots as a matrix for quantitative mercury analysis.

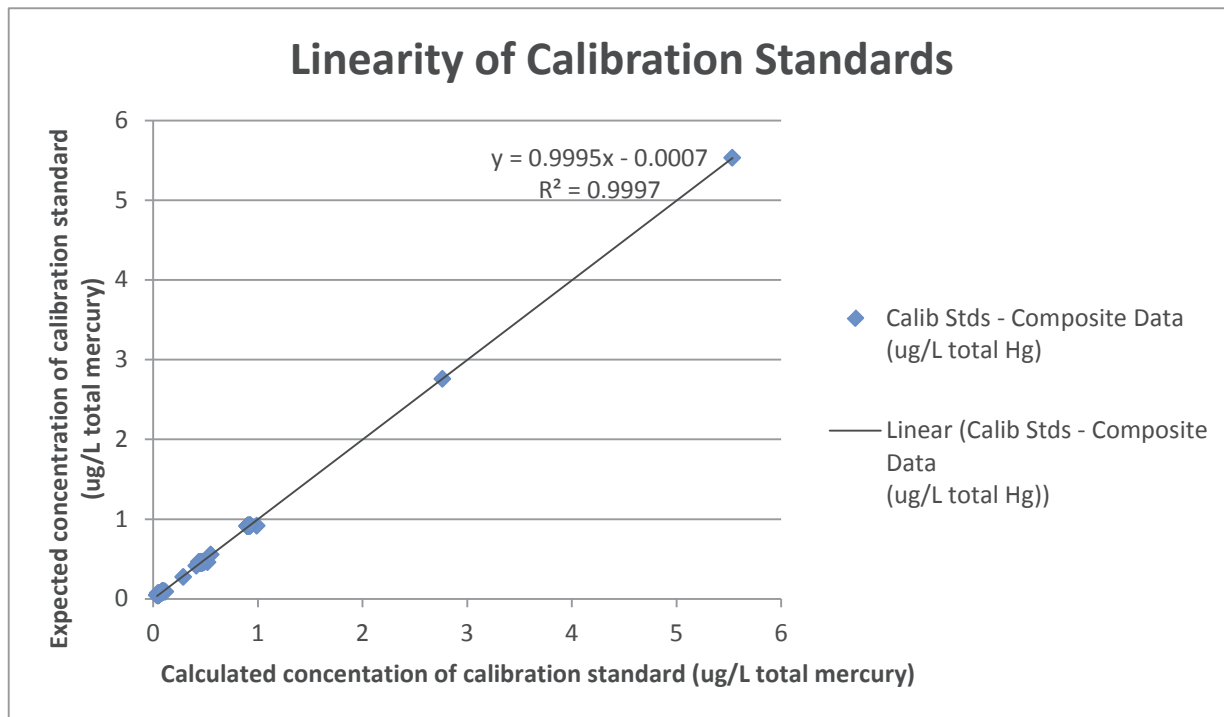
Linear dynamic range

One advantage for using the ICP-MS is its wide linear dynamic range, that is, the range over which the response of the instrument is linear with respect to analyte concentration. The slope of the line defined by the standards is proportional to the concentration in the standards. The unknown sample is run and its signal intensity is plotted against the curve to determine the concentration.

Prior to the start of the project, the Research Scientist that developed the method demonstrated linearity from the reporting limit of 0.046 ug/L to 5 ug/L total mercury, which corresponds to concentrations of 2.23 ug/L to 242 ug/L total mercury when analyzing two punches of dried blood spots, as was done in the project.

To demonstrate that the linearity was maintained throughout the course of the study and to demonstrate that the curve used to verify the high results was valid, the graph below was compiled using all calibration standards of the study.

Each daily calibration standard was reprocessed individually using the calibration curve produced on the day of analysis, then all of the observed values were plotted against the expected (true) value of the standard. There were 78 calibration data points for each of the five standards used routinely during the project and one data point for each of the six standards used to verify the high results.



A perfect correlation (R^2 value of 1.0 and the equality of the line being $y=x$) would demonstrate perfect linearity. While not perfect, the graph indicates very good linearity of the composited values over the course of the study. Typical correlation coefficients (R^2) for the calibration curves using CDC whole blood methods will be ≥ 0.999 .

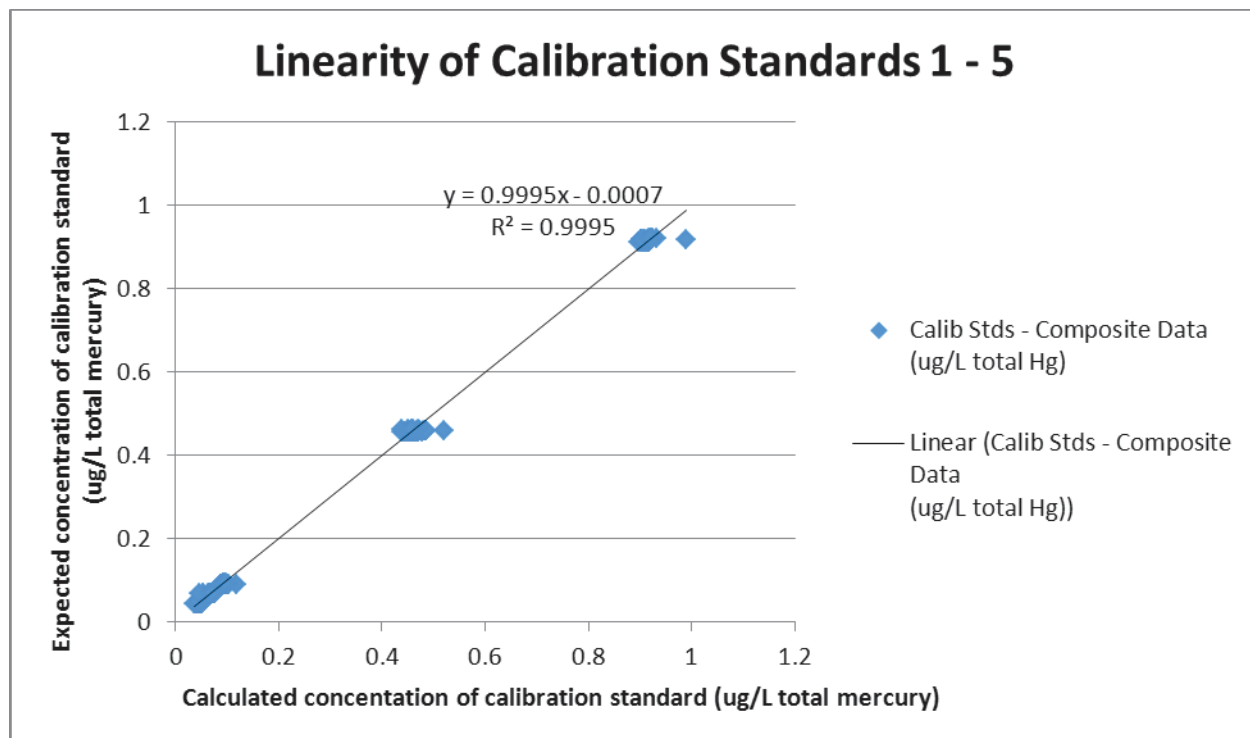
While the above graph indicates the linearity of all standards used, the higher standards were used only once during the study and only to verify that the value obtained from the routine standard calibration was valid.

There were 78 calibration data points for each of the five standards used routinely during the project. Due to the incorrectly assigned concentration values of stock methyl mercury concentration values, the concentrations of routine calibration standards (Standards 1 – 5) did not remain constant throughout the study.

The table below indicates the concentrations of Standards 1 – 5 that were used throughout the study.

Standard	Concentration (ug/L total Hg)	Number of data points
Standard 1	0.0455	62
	0.0458	10
	0.0461	6
Standard 2	0.0683	62
	0.0687	10
	0.0691	6
Standard 3	0.0911	62
	0.0915	10
	0.0922	6
Standard 4	0.456	62
	0.458	10
	0.461	6
Standard 5	0.911	62
	0.915	10
	0.922	6

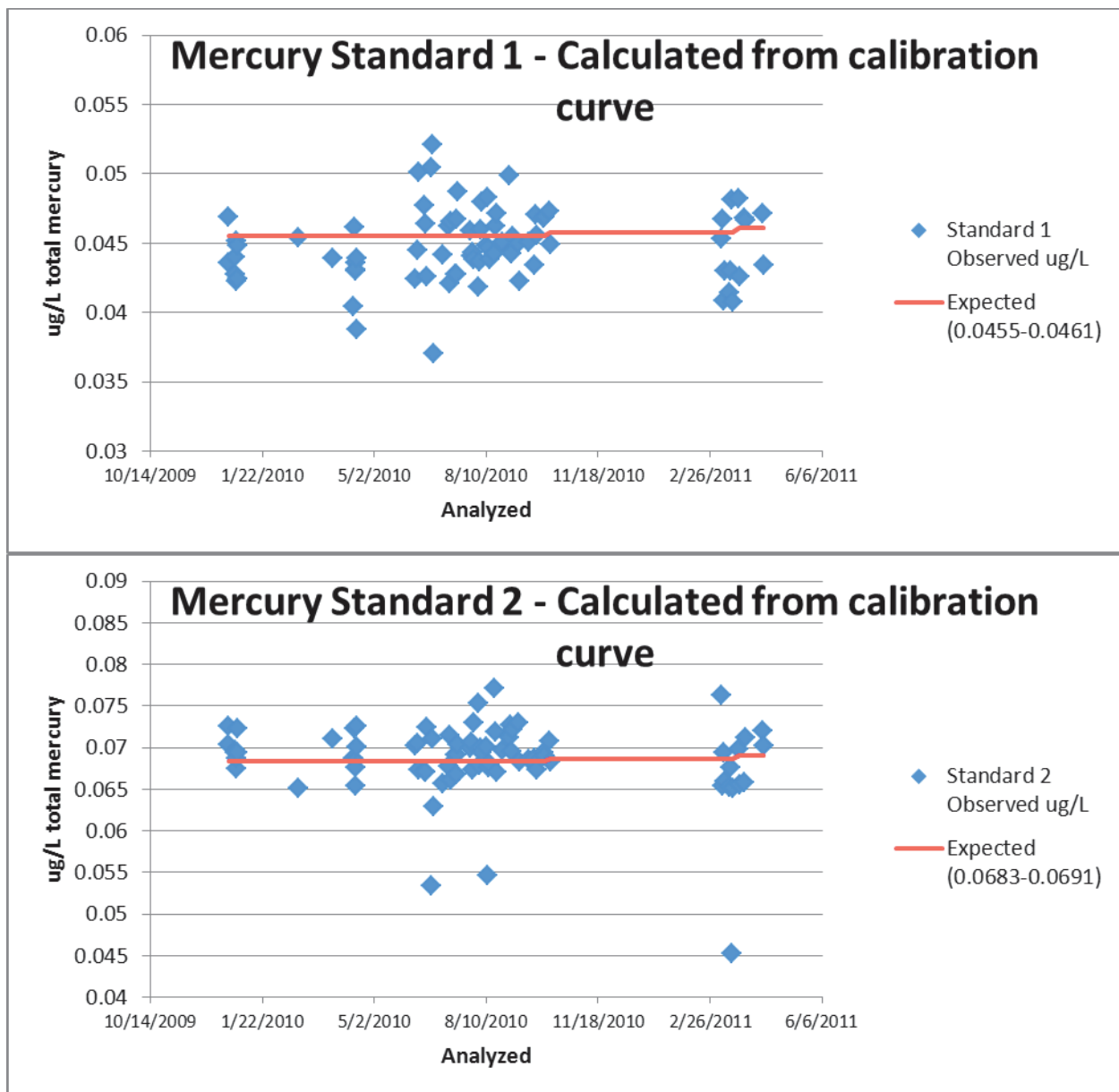
A similar graph to the one for all standards regarding linearity is presented below for the 78 data points for each of Standards 1 – 5.

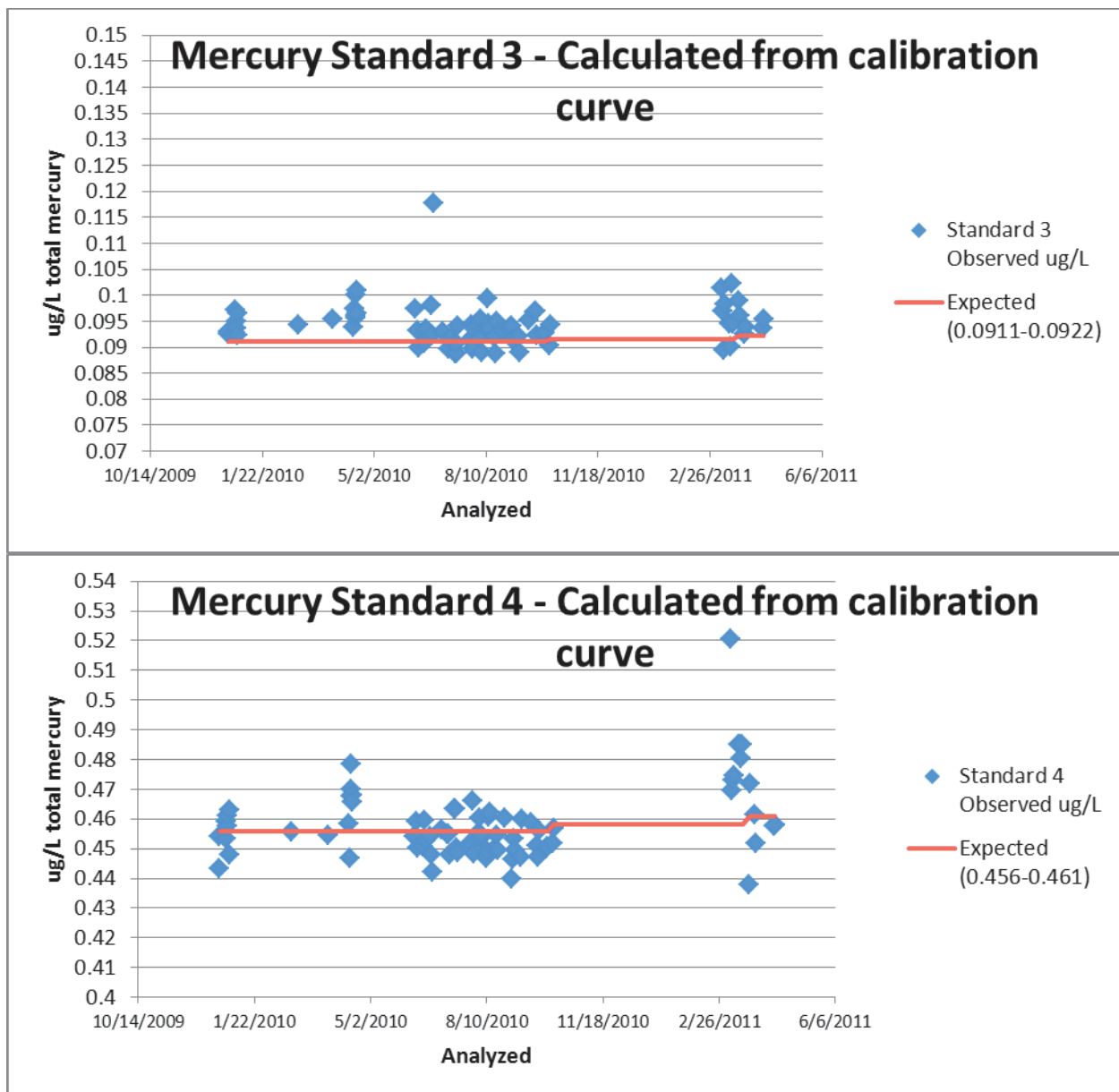


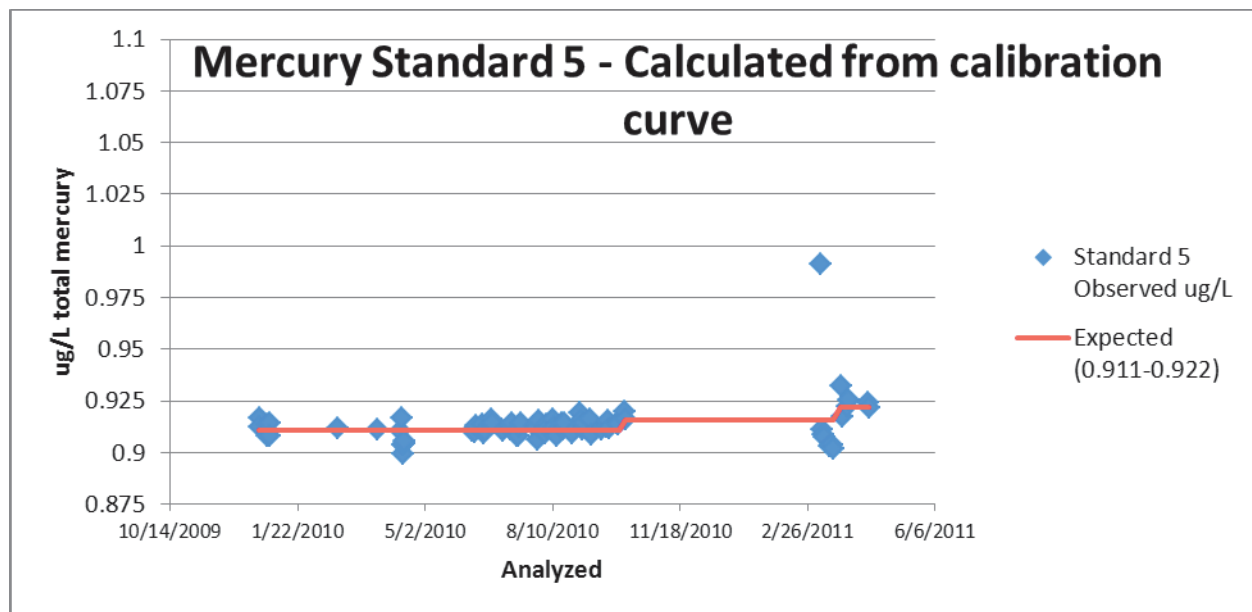
A perfect correlation (R^2 value of 1.0 and the equality of the line being $y=x$) would demonstrate perfect linearity. While not perfect, this graph also indicates very good linearity of the composited values over the course of the study.

Charts of Calibration Standards 1 – 5

The following charts show the observed values for the five calibration standards for the entire study. Each data point represents the value calculated from the curve when the standards were individually reprocessed.







Weaknesses of the method / areas of improvement

1. Standard Reference Material – availability of NIST-traceable materials

A prominent weakness of the method could be attributed to the laboratory's inability to obtain reference materials from more than one vendor.

The SOP indicates (Sec. 9.2.1) that calibration standards must always be traceable to the Nation Institute for Standards and Technology (NIST); however, the methylmercury standards obtained throughout the course of the study were not NIST-traceable. No other vendor could be found for the purchase of methylmercury standards.

Additionally, the laboratory misinterpreted the certificate of analysis provided by the vendor which caused the laboratory to ascribe an incorrect value for the methylmercury standard. Although this vendor's methylmercury standards are used in other Center for Disease Control and Prevention methods, when writing the initial release of the Mercury in Blood Spots by ICP-MS procedure, the MDH laboratory failed to address the need to convert the methylmercury chloride value to methylmercury.

Improvements:

- The laboratory's SOP should be revised to include the calculations necessary to convert the methylmercury chloride standard to methylmercury.
- Two vendors of NIST-traceable reference materials should always be used.
- The laboratory's SOP should be revised to include criteria for comparing the two vendor's standards.

2. Standard Reference Material – ratio of methylmercury to inorganic mercury

A 50:50 ratio of methylmercury to inorganic mercury was selected for calibration standards for the laboratory's methodology, without understanding how the mixture of the inorganic and organic forms of mercury would be extracted from dried blood spots.

Improvements:

- Further studies should be conducted to determine the extraction efficiencies of inorganic mercury, methylmercury, and varying ratios of the two.

3. Calibration Verification Standards – biased results evidenced

The calibration verification standards, despite being made from the same material as the calibration standards, indicated a high bias at the mid-level and high-level concentrations. No conclusive reason could be found to explain the high bias.

Improvements:

- Further studies should be conducted to assess all factors which might contribute to the high CVS bias.

4. Patient Samples – insufficient knowledge of volumes available prior to study's start

After initial estimates by the Newborn Screening Section staff on the availability of residual blood spots, it became evident that fewer "acceptable" spots were actually available on the cards of patients who qualified to be in the study.

Improvements:

- Future studies should be optimized to use two or fewer blood spots.

5. Punching Devices – insufficient attention to effort needed to hand punch samples

After initial estimates by the Newborn Screening Section staff on the effort it would take to punch residual blood spots by hand, it became evident that the staff of the Newborn Screening programs in the three states would not be able to fulfill their part of the study unless machine-punched specimens could be used.

Improvements:

- **Future studies should be optimized to use machine-punched specimens.**

6. Extraction Efficiency – lack of familiarity prior to study's start

Prior to analyzing samples, the laboratory intended to process samples by using four blood spots, 500 uL of diluent, and 450 uL of 2% hydrochloric acid.

As patient samples meeting the criteria for inclusion into the study were found to have insufficient blood available on the cards, the decision was made to decrease the number of blood spots and also to decrease the volume of reagents to maintain the method detection limit. Two blood spots from patient samples were analyzed in 150 uL of diluent and 150 uL of 2% hydrochloric acid.

The exact effect this reduced volume methodology on the ability to extract the mercury from blood spots was not known prior to presenting the measurement quality objectives (MQOs) for approval.

In addition, the MQOs were not based on a knowledge to extracting mercury from blood specimens, but rather from extracting mercury from aqueous samples. The assumption that the extraction efficiencies would match up well proved to be incorrect.

Improvements:

- **The extraction efficiency of known reference materials should be well studied prior to analysis of patient samples.**
- **The laboratory's SOP should be revised to reflect the true extraction efficiencies noted during the study.**



Please let me know if you have any questions or concerns with the issues in this report or any other issues regarding the laboratory's role in the study.

SSS

cc: Betsy Edhlund, Research Scientist 2, PHL
Jeff Brenner, Inorganic Chemistry Unit Supervisor, PHL
Paul Moyer, Environmental Laboratory Manager, PHL

Memo

DATE: November 29, 2011

TO: Patricia McCann, Principal Investigator and Project Manager
Environmental Health Division

FROM: Betsy Edhlund, Research Scientist
Public Health Laboratory Division (PHL)

PHONE: (651) 201-5302

SUBJECT: Quality Assurance Review of Mercury Data – Supplement II to Final Report
Mercury Levels in Blood from Newborns in the Lake Superior Basin
GLNPO Study ID 2007-942

This Supplement II to the Final QA report discusses any discrepancies from previous QA reports.

Additional information is provided on the following topics:

1. Specific errors from previous reports
2. Statements on accepting certain sample batches
3. A discussion on the relationship between aqueous-based and bloodspot mercury concentrations
4. A discussion on the recalculation of data based on the misrepresentation of the methyl mercury standard
5. A discussion regarding method improvements and weaknesses

Discrepancies from Final QA Report

The fourth paragraph on page 4, in the section on the reporting level verification, contains an incorrect date: April 30, 2011 should be changed to March 1, 2011. The sentence should read: One lot was used from December 23, 2009 to March 1, 2011 and was made from the same lot of standard as was used to produce the calibration standards on December 23, 2009.

In the fourth paragraph on page 5, the number of samples qualified due to a low recovery of the report level verification standard is incorrect. It should read: Of the 1496 patient samples analyzed, 98 samples (7% of all samples analyzed) were qualified due to a high recovery of the RLV standard and 80 samples (5% of all samples analyzed) were qualified due to a low recovery of the RLV standard.

On page 13, under the section describing the method blanks, there is an incorrect statement. It says that no method blank was observed with mercury concentration above the reporting limit. This is not true. There was one sample with a method blank of 3.93 µg/L, MN-0526. This sample was analyzed twice. The first analysis showed a method blank of < 2.13 µg/L, or below the reporting limit. However, the sample contained 66.6 µg/L mercury, which was outside of the calibration curve, and was therefore reanalyzed with an extended calibration curve. With the extended calibration curve, the sample had a mercury concentration of 66.9 µg/L and a method blank of 3.93 µg/L. Since the sample concentration is more than 10x the amount found in the blank and the blank concentration is below the low standard used for that batch (4.46 µg/L), the result was considered valid and reported.

Review of Batch MN-10

There is a discrepancy between the RLV reported for batch MN-10 and what was written in the QA report #1. The initial report states that the batch had a low RLV recovery (57%), when the actual reported recovery should be listed as 67%. Initially, and through April 2010, the RLV standard was analyzed twice per analytical run, although the QAPP requirements for only one RLV check per analytical run. After April 2010, the RLV was analyzed once per analytical run. For the batches that were analyzed with two RLV checks, the first RLV analyzed was reported. For batch MN-10, the second RLV was reported in the QA report instead of the first, which was within the QA limits.

New CVS Limits for Accepting MI-01 and MI-02

New standards were prepared on October 4, 2010. These new standards were used to analyze batches MI-01 and MI-02 on October 6 and 7, 2010, respectively. The continuous calibration verification samples did not meet the acceptance criteria. All further batches were halted and new acceptance criteria limits were established for the new lot of material used on November 5, 2010. Batched MI-01 and MI-02 were then deemed acceptable based on the new CVS limits and the results were reported.

Accepting Samples Based on CVS Failures

The calibration curve for each batch was verified through the use of calibration verification standards (CVS). Each batch contained three sets of CVSs at three levels (low, medium, and high). Patient samples were bracketed by CVS pairs and the average of the pairs was used to evaluate the acceptability of the calibration. During the course of the study, the results from a handful of batches were not initially reported due to CVS failure. All rejected samples were held until the completion of the initial analysis of every sample before reanalysis. Before reanalysis was started, each of the rejected data was reviewed. It was determined that for batches with a partial calibration verification, sample results falling within the verified portion of the calibration would be reported and qualified. Samples falling outside of the verified portion were reanalyzed. An example would be a batch with the low and medium CVS passing and failing high CVS. In this case, any samples with a concentration below the medium CVS level would be qualified and reported and any samples above the medium CVS would be reanalyzed. The QAPP for this project should have been revised to include this data rejection amendment.

Accepting Samples Based on QCS/LCS Recoveries

The acceptance criteria published in the QAPP for both the QCS (NIST standard reference material for mercury in blood) and the LCS (NIST SRM spotted onto filter paper cards) is stated as 80 – 120% recovery. Initially, any batches with QCS and/or LCS recoveries falling outside this range were to be reanalyzed. Throughout the study it was determined that the average percent recovery for the QCS was 86% and 81% for the LCS. Using the average $\pm$ 3-sigma, a new acceptable range for each of these standards was developed: 70 – 101% for the QCS and 64 – 98% for the LCS. These new limits take into account the extraction efficiency of the method. It was decided that any batches with QCS and LCS recoveries that would have failed the QAPP guidelines while passing using the 3-sigma ranges would be reported and qualified. Any samples with corresponding QCS and LCS outside of the 3-sigma ranges would be reanalyzed.

Concentrations in Blood versus Aqueous Standards

The calibration curve standards, blanks, and continuous calibration verification (CVS) samples were aqueous-based. All results reported from the instrument were based on the concentrations of these aqueous-based samples. To convert a concentration from aqueous-based to the amount of mercury found in the blood, the following formula is needed:

$$\text{Hg concentration in blood} = \frac{\text{aqueous-based concentration } (\mu\text{g/L}) \times \text{volume in well-plate } (\mu\text{L})}{\text{volume of blood in two bloodspot punches } (\mu\text{L})}$$

$$\text{Hg concentration in blood } (\mu\text{g/L}) = \frac{\text{aqueous-based concentration } (\mu\text{g/L}) \times 300 \mu\text{L}}{6.2 \mu\text{L}}$$

Recalculation of Data Based on Misrepresentation of Standard Concentration

All of the reported sample concentrations were recalculated due to a discovered discrepancy between the actual concentration of methyl mercury and the presumed concentration from the certificate of analysis in each lot of standard used throughout the study. This discrepancy arose from our belief that the concentration listed on the certificate of analysis was the concentration of mercury, when in fact it is listed as the concentration of methyl mercury chloride. After obtaining the exact concentration of mercury for each lot of standard used from the manufacturer, each calibration curve, and subsequently each sample, was reprocessed to reflect the correct concentration of mercury present in each standard. This was accomplished by calculating the correct mercury concentration in each standard, entering that concentration into the method in the ICP-MS software, and reprocessing each batch of data against the newly determined calibration curve. This recalculation resulted in a change in the method MDL and report level. The revised MDL is 0.70 $\mu\text{g/L}$ and the report level was adjusted to 2.13 $\mu\text{g/L}$. Since two lots of methyl mercury standard were used to make the report level verification sample, the percent recoveries reported for the RLV are calculated based on which lot of material was used, for a true value of either 2.13 $\mu\text{g/L}$ or 2.21 $\mu\text{g/L}$.

Weaknesses of the method / areas of improvement

There are a few weaknesses to this method with some areas for improvement, but there also are some strengths as well. One weakness is the availability of standards, both the methyl mercury standard and a certified reference material in a suitable concentration range. Having one vendor produce a methyl mercury standard is what lead to the discrepancy in



the reported concentration. If more vendors were available, two different sources of materials would have been used, and the discrepancy would have been discovered much sooner and accounted for from the beginning. There are now more vendors available, so if this method were to be used again, there would need to be a change in the SOP to require the use of two separate sources of material and to correct the vendor concentration to be that of methyl mercury, not methyl mercury chloride, as was our mistake.

Also related to available standards, would be to find a certified reference material with a mercury concentration closer to that of the expected sample concentrations. In this case, the available SRM from NIST had a mercury concentration of 31.4 µg/L, which is well above the general concentrations observed in samples. However, there is a new SRM available from NIST that is certified for mercury at a concentration of 17.8 µg/L and has a reference value of 4.95 µg/L in another level. While these levels are still higher than most seen in the study, they are much closer than the original SRM.

Another weakness of this method is the limited amount of sample that was available for use. This hindered the method in a couple of ways. First, the limited amount of sample greatly impacted the volume of sample that could be used for analysis. Currently, this method uses two bloodspot punches with a total blood volume of 6.2 µL. If more sample was available, this volume could be increased having a significant impact on lowering the method detection and reporting limits and allowing for more samples to be reported above the detection limit and without being estimated below the reporting limit.

More available sample would also reduce the number of qualified samples. For this study, the laboratory received enough of each sample for two analyses. If there was a QA failure, there was only one more chance to reanalyze. Connected with this, is that every sample analyzed with a batch, the calibration standards, all QA samples, and patient samples, were prepared together, allowed to sit overnight and then analyzed. The upside to this approach is that every sample is treated the same way; they all sit in the same conditions and are filtered at the same time. The downside to this approach is that with this particular method the whole batch must be run with no chance to re-inject a sample. Each prepared sample is only enough volume for one injection and cannot be re-prepared for analysis with the current batch. While it seems that there are a high number of qualified results, they appear to be on-par with other methods run by the laboratory, the difference is that with those other methods there are other opportunities for reanalysis so that the QA issues get resolved.

While this method has its challenges, it is also very consistent. In general, the recoveries for the report level verification standards, the QCS, LCS, and reference samples all were very consistent over the course of the study, approximately 1 ½ years. Also, with such a limited amount of sample, this method may be better suited for a qualitative, screening-



type study in which the researcher is looking for elevated levels of mercury and is unconcerned with baseline concentrations. A follow-up analysis could then be performed using a whole blood sample collected from the exposed individual.

Please let me know if you have any questions or concerns with the issues in this report or any other issues regarding the laboratory's role in the study.

BLE

cc: Jeff Brenner, Inorganic Chemistry Unit Supervisor, PHL
Paul Moyer, Environmental Laboratory Manager, PHL

Tribes in Minnesota are paying the steepest price for the steel industry's mercury pollution

Steel companies that process taconite release a slew of pollutants — and they're suing the EPA over new regulations.



Clearing out the bog growth at Big Rice Lake in Minnesota. **Joey Mcleister / Star Tribune via Getty Images**

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Demand for steel is on the rise globally, driven by population growth and the expanding economies in developing nations. The material will also be important to the green energy transition, forming the backbone of infrastructure like wind turbines, solar panels, and hydroelectric dams. Every part of the steel supply chain is heavily polluting, and the places in the U.S. where the steel industry is concentrated are disproportionately low-income and nonwhite, highlighting yet another instance in which the promises of development and climate solutions come at a steeper cost for some communities. What’s more, the country’s steel production is dominated by just two companies: U.S. Steel and Cleveland Cliffs.

For both companies, much of their production begins with taconite, a low-grade iron ore mined in the northeast Minnesota’s Mesabi Iron Range, which is processed into pellets that get shipped to the steel mills of Gary, Indiana. The extraction of the ore from taconite rock releases a slew of toxic pollutants into the air, including mercury, lead, and dioxins. In this region, the most



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Studies have connected mercury to a litany of negative health effects. It's a neurotoxin that can interfere with brain development in unborn children and an endocrine disruptor that can weaken the immune system. Scientists have yet to determine a quantity of mercury that is safe for human consumption. One recent study found that there is "no evidence" for a threshold "below which neuro-developmental effects do not occur." And while the taconite industry releases less than a ton of mercury into the atmosphere every year, the metal is toxic in extremely small quantities: A fraction of a teaspoon can contaminate a 20-acre lake.

The nation's six taconite plants, all in this region of Minnesota, are owned by U.S. Steel and Cleveland Cliffs. In May 2023, the Environmental Protection Agency proposed a regulation that would require the companies to cut their mercury emissions by around 30 percent. In order to meet that standard, the companies would have to install equipment that would inject carbon atoms into their industrial chimneys so that the carbon would attach itself to the mercury atoms, making the pollution particles bigger and allowing them to get trapped in a filter before they would be released into the atmosphere. The agency estimates that its regulation would cost the industry \$106 million in capital costs and \$68 million per year thereafter.

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Last month, when the standards were finalized, both companies sued. They argue that the regulation would pose “irreparable harm” to the industry, because of the steep costs of implementation. They also argue that the EPA’s proposed method for reducing mercury pollution would actually be worse for public health, causing a 13 percent *increase* in the amount of the toxic metal deposited in the local environment.

“EPA is not only requiring industry to restructure its operations and build new pollution-control facilities at unprecedented costs, it is requiring facilities to commit to associated disruption of their current operations, spend hundreds of millions of dollars, and risk their productive capacity and, indeed, ability to operate

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completely, to design, permit, and install a technology with no demonstrated ability to actually work,” the companies wrote.

Jim Pew, a lawyer at Earthjustice who has litigated multiple lawsuits against the EPA for its failure to curb pollution from the taconite industry, pointed out that the costs of implementing the required equipment would be a tiny fraction of the companies’ annual sales, which totaled \$40 billion in 2023. Pew noted that U.S. Steel recently initiated a \$500 million stock buyback program, the mark of a healthy income revenue stream. As for the companies’ claim that the technology would increase mercury pollution, Pew called it “meritless.” The companies are “relying on a premise they know to be false” — that taconite plants would add the carbon technology without also improving their filtration system.

“I find this reprehensible and shameful,” Pew said. “While it’s claiming that it can’t spend money to clean up historic pollution, U.S. Steel is just handing out money to its shareholders.”

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In an email, a spokesperson from U.S. Steel told Grist that the company's lawsuit was meant to ensure that the EPA's new regulations are "in line with sound science and regulatory procedures." The spokesperson went on to say that the company had tested the available emissions-reduction technology at one of their plants in Minnesota and determined that it would not be in compliance with the mercury limits established by the agency. "We remain committed to environmental excellence, as do the nearly 2,000 hardworking men and women of our Minnesota Ore Operations." Cleveland Cliffs did not respond to multiple requests for comment.

Pew sees the lawsuit as part of a multipronged attack by the steel industry against federal regulation. Over the past several years, the EPA has also proposed standards for the other types of facilities involved in steel production. These two companies have threatened litigation at every turn, recently petitioning a bipartisan group of lawmakers to send a letter to EPA Administrator Michael Regan, asking him to loosen the new standards for steel mills.

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Taconite is dumped from railroad cars in Minnesota, 1965. *Minnesota Historical Society via Getty Images*

By the terms of the Clean Air Act, the EPA was supposed to propose standards to control toxic releases from taconite plants in 2003. When they failed to do so, environmental advocates from the Save Lake Superior Association and other groups sued the following year. In a federal circuit court, the EPA acknowledged that it had fallen short of its duties and promised to move with “all due process and speed” to fill the gaps in its regulations.

Years passed without a federal rule, and in 2007, Minnesota initiated an effort of its own, setting a standard for mercury pollution in water and, two years later, becoming the first state to develop a plan to achieve it. The standard required industries across the state to slash their emissions by a cumulative 93 percent, and over the following decade, power plants, crematoria, and other mercury emitters achieved major reductions. Emissions from the taconite industry, however, remained exceptionally high.

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Mercury contamination is particularly worrisome for tribal nations like the Fond du Lac Band, which fish and grow wild rice throughout the state's vast network of rivers, lakes, and streams. "We find that across a lot of ceded territory, there's a lot of good regulation but there's been a lot of flexibility in enforcement," said John Coleman, an environmental scientist at the Great Lakes Indian Fish and Wildlife Commission.

Tribes repeatedly petitioned the EPA to make good on its 2003 promise. They had good reason to be concerned: One study had found that 10 percent of babies born on the north shore of Lake Superior have elevated mercury levels in their blood.

It took the agency until last May to finally propose its regulation, which, of course, is under challenge. Still, for the tribes of northeast Minnesota, the EPA's rule was a resounding disappointment. Even if U.S. Steel and Cleveland Cliffs reduce their mercury emissions by 30 percent, the companies' operations would still allow hundreds of pounds of mercury to enter the state's waterways each year.

"It is of our view that these proposed standards do not go far enough toward restoring and protecting the health and well-being of the environment and our community," wrote Paige Huhta, the Fond du Lac's air program coordinator in a letter to the EPA last July. She pointed out that the EPA itself had found that exposure among specific subpopulations, including some tribes, may be more than twice as great as that experienced by the average American. But when the agency finalized the rule this past March, it did not budge from its original reduction requirements.

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“Water is an important part of the landscape up here,” said Nancy Shuldt, the Fond du Lac Band’s Water Projects Coordinator. “We have a water-rich landscape and water resources form the foundation of tribal lifeways.”

And because it is a metal, mercury does not break down into less toxic substances like other industrial pollutants. It stays in the environment for hundreds of years. In northeastern Minnesota, and to a specific group of people, much of the damage has already been done.

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Nippon Steel Corporation and U. S. Steel Finalize Historic Partnership

JUNE 18, 2025

7 Minutes Read

*U. S. Steel to Retain Its Iconic Name and Headquarters in Pittsburgh,
Pennsylvania*

and Continue to Be Mined, Melted, and Made in America

Partnership Expected to Protect and Create More Than 100,000 Jobs

*Moving Forward Together as the 'Best Steelmaker with World-Leading
Capabilities'*

Tokyo and Pittsburgh – June 18, 2025 – Nippon Steel Corporation (TSE: 5401) (“Nippon Steel”), together with its wholly owned subsidiary Nippon Steel North America, Inc. and United States Steel Corporation (“U. S. Steel”) (collectively, the “Companies”), today announced they have finalized their historic partnership.

Together, Nippon Steel and U. S. Steel will be a world-leading steelmaker, with best-in-class technologies and manufacturing capabilities. The partnership will protect and create more than 100,000 jobs through unprecedented massive investments in steelmaking in the United States that will be unleashed across U. S. Steel’s footprints, including in Pennsylvania, Indiana, Arkansas, Minnesota and Alabama. This partnership ensures that U. S. Steel will retain its iconic name and headquarters in Pittsburgh, Pennsylvania, and that it will continue to be Mined, Melted, and Made in America for generations to come.

Eiji Hashimoto, Nippon Steel’s Representative Director, Chairman and CEO, said, “I am very pleased that the partnership between Nippon Steel and U. S. Steel has been realized thanks to President Trump’s historic and visionary decision. I would like to thank the wide range of stakeholders in the United States and Japan, including U. S. Steel employees, local communities, government officials, elected officials, and business leaders, for their endorsement and support of this partnership. Nippon Steel is excited about opening a new chapter of U. S. Steel’s storied history. Building on our investment, the transfer of our advanced technologies, and the unwavering efforts of management and the employees of both companies, Nippon Steel is committed, together with U. S. Steel, to solidifying its position as the world’s leading steelmaker.”

Takahiro Mori, Nippon Steel’s Representative Director and Vice Chairman, and who will serve as Chairman of the Board of U. S. Steel said, “Since announcing our deal, I have engaged in extensive dialogue with many stakeholders, including the employees of U. S. Steel, government officials and community leaders, and I would like to express my sincere gratitude to all of them for their tremendous cooperation and support for this partnership. I am delighted that we have made this day a reality. We share President Trump’s commitment to protect the future of the American steel industry, American workers, and American national security, and we look forward to building a stronger and brighter future for U. S. Steel.”

U. S. Steel’s CEO Dave Burritt stated: “This is a momentous day for our country, our communities, and the American steel industry. Thanks to President Trump’s bold leadership, American workers secured the best possible deal. U. S. Steel will

remain rooted in the United States and continue to call Pittsburgh home. Through our partnership with Nippon Steel, we are poised to grow better and bigger, with transformative investment, cutting-edge technology, and the creation of good-paying jobs across the United States.”

The Companies have now completed the transaction as contemplated by their merger agreement. The Companies have also entered into a National Security Agreement (“NSA”) with the U.S. Government, and U. S. Steel will issue a Golden Share to the U.S. Government.

Under the NSA, the U.S. Government and the Companies have agreed to measures to protect U.S. national security, including, among others, the following commitments by the Companies:

- **Investment** - Nippon Steel will make approximately \$11 billion in new investments in U. S. Steel by 2028, which includes an initial investment in a greenfield project that will be completed after 2028;
- **U.S. Headquarters** - U. S. Steel will remain a U.S.-incorporated entity and will maintain its headquarters in Pittsburgh, Pennsylvania;
- **U.S. Board** - A majority of the members of U. S. Steel’s board of directors will be U.S. citizens;
- **U.S. Management** - U. S. Steel’s key management personnel, including its CEO, will be U.S. citizens;
- **U.S. Production** - U. S. Steel will maintain capacity to produce and supply steel from its U.S. production locations to meet market demand in the U.S.; and
- **U. S. Steel Autonomous Trade** - Nippon Steel will not prevent, prohibit, or otherwise interfere with U. S. Steel’s ability to pursue trade action under U.S. law

Pursuant to the NSA and through the ownership of the Golden Share, the U.S. Government will have certain rights, including, among others:

- The right to appoint one independent director; and
- Consent rights of the President of the United States, or his designee, on specific matters, including:
 - Reductions in the committed capital investments under the NSA;
 - Changing U. S. Steel’s name and headquarters;
 - Redomiciling U. S. Steel outside of the United States;
 - Transfer of production or jobs outside of the United States;
 - Material acquisitions of competing businesses in the United States; and
 - Certain decisions on closure or idling of U. S. Steel’s existing U.S. manufacturing facilities, trade, labor, and sourcing outside of the United States.

This framework will enable Nippon Steel to secure management flexibility and profitability at U. S. Steel while protecting U.S. national security.

With this partnership, the Nippon Steel Group’s annual crude steel production capacity is expected to reach 86 million tons, bringing it closer to Nippon Steel’s global strategic goal of 100 million tons of global crude steel production capacity.

The total number of jobs that will be created and is referenced above is based on a study done by Parker Strategy Group, commissioned by U. S. Steel. Total jobs figure includes direct, indirect and induced jobs.

Advisors

Citi acted as financial advisor to Nippon Steel. Ropes & Gray LLP acted as legal advisor to Nippon Steel. PJT Partners and Akin Gump Strauss Hauer & Feld LLP acted as strategic advisors to Nippon Steel. Barclays Capital Inc., Goldman Sachs & Co. LLC and Evercore acted as financial advisors to U. S. Steel. Milbank LLP, Covington & Burling LLP and Wachtell, Lipton, Rosen & Katz acted as legal advisors to U. S. Steel.

About Nippon Steel

Nippon Steel is one of the world's leading steelmakers and Japan's largest steelmaker. Nippon Steel has a global crude steel production capacity of approximately 86 million tonnes and employs around 136,000 people in the world. Nippon Steel's manufacturing base encompasses more than 15 countries including: Japan, the United States, India, Thailand, Indonesia, Vietnam, Brazil, Mexico, Sweden and others. Nippon Steel established a manufacturing base in the United States around 40 years ago and has focused on building cooperative and good relationships with employees, labor unions, suppliers, customers, and communities. As the 'Best Steelmaker with World-Leading Capabilities,' Nippon Steel pursues world-leading technologies and manufacturing capabilities and contributes to society by providing excellent products and services. For more information, please visit: <https://www.nipponsteel.com>.

About U. S. Steel

Founded in 1901, U. S. Steel delivers profitable and sustainable steel solutions. Propelled by its talented employees and an unwavering focus on safety, U. S. Steel serves the automotive, construction, appliance, energy, containers, and packaging industries with high value-added steel products. Steel production begins with our competitively advantaged iron ore production capabilities which fuel our integrated steelmaking facilities and investments in electric arc furnaces. To help our customers create the best products with the fewest emissions, we are committed to reaching net-zero greenhouse gas emissions by 2050. U. S. Steel is at the forefront of creating steels that are stronger, lighter, and better for the environment. This includes our proprietary XG3® advanced high-strength steel, verdeX® steel produced with 70-80% lower CO2 emissions with a recycled content of up to 90%, and ultra-thin lightweight InduX™ steel for electric vehicles, generators, and transformers. U. S. Steel maintains operations across the United States and in Central Europe and is headquartered in Pittsburgh, Pennsylvania. For more information, please visit www.ussteel.com and follow U. S. Steel on LinkedIn, Instagram, Facebook, and X.

CAUTIONARY NOTE REGARDING FORWARD-LOOKING STATEMENTS

This press release contains information regarding U. S. Steel and Nippon Steel that may constitute "forward-looking statements," as that term is defined under the Private Securities Litigation Reform Act of 1995 and other securities laws, that are subject to risks and uncertainties. We intend the forward-looking statements to be covered by the safe harbor provisions for forward-looking statements in those sections. Generally, we have identified such forward-looking statements by using the words "believe," "expect," "intend," "estimate," "anticipate," "project," "target," "forecast," "aim," "should," "plan," "goal," "future," "will," "may" and similar expressions or by using future dates in connection with any discussion of, among other things, statements expressing general views about trends, events or developments that we expect or anticipate will occur in the future, potential changes in the global economic environment, anticipated capital expenditures, the construction or operation of new or existing facilities or capabilities and the costs associated with such matters, as well as statements regarding the transaction. However, the absence of these words or similar expressions does not mean that a statement is not forward-looking. Forward-looking statements include all statements that are not historical facts, but instead represent only U. S. Steel's and Nippon Steel's beliefs regarding future goals, plans and expectations about our prospects for the future and other events, many of which, by their nature, are inherently uncertain and outside of U. S. Steel's or Nippon Steel's control and may differ, possibly materially, from the anticipated events indicated in these forward-looking statements. Management of U. S. Steel or Nippon Steel, as applicable, believes that these forward-looking statements are reasonable as of the time made. However, caution should be taken not to place undue reliance on any such forward-looking statements because such statements speak only as of the date when made. In addition, forward looking statements are subject to certain risks and uncertainties that could cause actual results to differ materially from U. S. Steel's or Nippon Steel's historical experience and our present expectations or projections. Risks and uncertainties include without limitation: the risk that any announcements relating to the transaction could have adverse effects on the market price of Nippon Steel's common stock or American Depositary Receipts; the risk of any unexpected costs or expenses resulting from the transaction; and the risk that the transaction could have an adverse effect on the ability of U. S. Steel or Nippon Steel to retain customers and retain and hire key personnel and maintain relationships with customers, suppliers, employees, stockholders and

MCEA Exhibit 5

other business relationships and on its operating results and business generally. U. S. Steel directs readers to its Form 10-K for the year ended December 31, 2024 and Quarterly Report on Form 10-Q for the quarter ended March 31, 2025, and the other documents it files with the SEC for other risks associated with U. S. Steel's future performance. These documents contain and identify important factors that could cause actual results to differ materially from those contained in the forward-looking statements.

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MINING

What the U.S. Steel sale means for Minnesota's Iron Range

Part of the Nippon Steel's national security agreement with Trump administration includes \$800M in local investments, few job protections.

Jerry Burnes

June 19, 2025 4 minutes



U.S. Steel officials talk with Takahiro Mori, a Nippon Steel executive, in May 2024 at a ribbon cutting event for Keewatin Taconite. (Jerry Burnes/Iron Range Today)

Nippon Steel [completed its purchase of U.S. Steel on Wednesday](#), placing two Iron Range mines under new ownership with some oversight from the federal government under a national security pact.

The deal includes a “golden share” that gives President Donald J. Trump and future administrations an ability to block certain actions and some leverage to enforce promises made by the Japanese steelmaker.

In practice, the arrangement is a permanent single share of preferred stock in U.S. Steel that allows the president or a designee to effectively serve on the company’s board.

As part of the pact, a number of potential activities by the company are disallowed without approval of the president, ranging from moving its headquarters to following through on investments.

ALSO READ: [*A look at the 'golden share' agreement in the U.S. Steel-Nippon Steel partnership \(NPR News\)*](#)

For the Iron Range, the deal intends to send about \$800 million in investments to U.S. Steel mines between 2025 and 2028 as part of a larger promise to spend \$11 billion domestically. That total also calls for construction of a new \$1 billion mini mill at an undetermined location.

Nippon is set to spend \$200 million in 2025 and 2026, and \$300 million in 2027 and 2028 at the company's Minntac and Keetac facilities, subject to approvals and permits, [according to SEC filings](#).

No details have been provided on the scope of those local investments, and a spokesperson for U.S. Rep. Pete Stauber told Iron Range Today recently that particulars were still unknown.

The United Steelworkers (USW) have been the biggest opponent of the merger since it was announced in December 2023, and supported U.S. Steel rival Cleveland-Cliffs to acquire the company.

Union officials were miffed that the company didn't consult with them before agreeing to the Nippon Steel sale, and have sought written assurances from the new parent company that would protect contracts, jobs and retiree benefits.

Nippon has said it would honor current collective bargaining agreements with USW, which are set to expire next year, but little-to-no language concerning workers and jobs was present in the national security pact publicized Wednesday.

The deal calls for the company to maintain base salaries of current employees until 2030, and is meant to safeguard against plant closures, idles or sales up to June 18, 2035, including at Minntac and Keetac.

But it also carved out an exception for temporary plant idles, which typically include layoffs and a more open-ended timeline than the term implies.

That's the [current case at two Cliffs facilities on the Iron Range](#). The company announced temporary idles in March, [impacting 630 miners at Hibbing Taconite and Minorca Mine](#).

Company officials anticipated a six-month down period, but union leaders have expressed concern over the winterizing process at Minorca – calling it instead an “indefinite idle” period, and whether the full workforce at HibTac will ever return.

[USW President Dave McCall said Wednesday](#) that union would work to hold Nippon responsible to its promises.

“Our current agreement expires in September 2026,” he said, “and rest assured, if our job security, pensions, retiree health care or other hard-earned benefits are threatened, we are ready to respond with the full strength and solidarity of our membership.”

More broadly, the Keetac and Minntac facilities would stand to benefit from a cash infusion.

Keetac [recently finalized upgrades to produce of DR-grade pellets](#) to feed electric-arc furnaces, and was reportedly a facility of interest for Nippon from the start. It also faces [a large unknown with its water permits and state sulfate standards](#), which could present a significant cost hurdle.

Minntac also has permit renewals on the horizon, as well as general maintenance at the largest taconite mine in the United States.

Nippon will also have to navigate a 25% ownership stake in Minntac by Cliffs, [stemming from a pandemic-era decision by U.S. Steel to sell part of the mine to Stelco in 2020](#). Terms of the deal appear to allow the company a clean out in 2027, but at a cost of \$120 million. Stelco was [purchased by Cliffs](#) last year.

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TOP STORY

A massive investment in Minnesota's Iron Range

By LEE BLOOMQUIST FOR MESABI TRIBUNE

May 28, 2025

All the components of a United States Steel Corp. and Nippon Steel Corp. partnership still aren't clear.

But it appears a combination of the two steelmakers could hold nearly \$1 billion of dollars of investment in U.S. Steel's Iron Range facilities.

"It appears the investment on the Range is going to be significant," Minnesota Eighth District U.S. Congressman Pete Stauber said. "The partnership between Nippon Steel and U.S. Steel will revive America's steel industry, and the benefits will be felt right here in northern Minnesota. This deal will inject \$14 billion into the U.S. economy, and this includes an estimated \$800 million investment into the Iron Range. Make no mistake, this is a significant investment that will create jobs and strengthen our region's proud iron mining legacy."

Nippon Steel previously said it would invest \$2.7 billion in U.S. Steel facilities, including the Mon Valley Works in Pennsylvania and Gary Works in Gary, Ind.

But Nippon Steel has now upped its total investment commitment to \$14 billion, according to President Donald Trump.

An \$800 million investment by Nippon Steel Corp. into U.S. Steel's Minnesota Ore Operations facilities at Minntac Mine in Mountain Iron and Keetac in Keewatin would be huge news for the taconite plants and workers at the facilities.

Minntac Mine began operations in 1967.

Keetac opened in 1967 as National Steel Pellet Co. and in 2003 was acquired by U.S. Steel.

Minntac Mine and Keetac employ more than 1,800.

Neither U.S. Steel or Nippon Steel have outlined details of a partnership.

However, Trump said the U.S. government would control U.S. Steel as part of the deal.

“It will be controlled by the United States, otherwise I wouldn’t make the deal,” Trump said.

To help push the deal through, Nippon Steel in 2024 offered to give the U.S. government veto power over any reduction of U.S. Steel’s production capacity.

A majority of a board of directors would be composed of U.S. Steel board members, according to reports.

Stauber said he and other federal legislators met with Trump on Thursday on the issue.

“Generations of Minnesota’s miners have mined the iron ore to make the American steel that meets our national security, infrastructure, and manufacturing needs,” Stauber said. “Our steel industry has taken some hits, but this partnership will strengthen and reinvigorate the American steel industry, allowing Minnesota’s miners to continue this proud heritage. I met with President Trump on Thursday on this matter, and I applaud him for his quick and decisive leadership.”

Locally, United Steelworkers (USW) leaders say they haven't received any information on the announced partnership.

"I have heard nothing," Steve Bonach, USW Local 1938 president at Minntac Mine said. "It's been kind of silent all the way around except for what Trump said and CFIUS (Committee on Foreign Investment in the United States.)"

The USW International has opposed a deal between the two steelmakers since it was announced more than a year ago.

"I oppose it totally," Bonach said. "It's too vague with our BLA (Basic Labor Agreement). I don't agree with it at all."

Jake Friend, USW Local 2660 president at Keetac said he hasn't received any information on the announced partnership.

"We haven't heard anything," Friend said. "I wish I had answers, but I really don't know any details on it."

Meantime, Minntac Mine continues to operate as usual, Bonach said.

"We go about our business and that's the way it is," Bonach said. "We're waiting for something to burst."

Reports are that of the \$14 billion, Nippon Steel would invest \$4 billion in a green field steel mill.

Nippon Steel officials have maintained that a full acquisition of U.S. Steel has been its goal.

But Trump is calling it a partnership.

Trump said Minnesota would be part of Nippon Steel's investment.

"I am proud to announce that, after much consideration and negotiations, US Steel will REMAIN in America, and keep its Headquarters in the Great City of Pittsburgh," Trump said on Truth Social. "For many years, the name "United States Steel" was synonymous with Greatness, and now it will be again. This will be a planned partnership between United States Steel and Nippon Steel, which will create at least 70,000 jobs and add \$14 billion dollars to the U.S. Economy. The bulk of that investment will occur in the next 14 months. This is the largest Investment in the History of the Commonwealth of Pennsylvania. My Tariff Policies will ensure that Steel will once again, be, forever, MADE IN AMERICA. From Pennsylvania to Arkansas, and from Minnesota to Indiana, AMERICAN MADE is BACK. I will see you all at US Steel in Pittsburgh on Friday, May 30, for a BIG rally. CONGRATULATIONS TO ALL!

In response, U.S. Steel called Trump "a bold leader and a businessman who knows how to get the best deal for America, American workers and American manufacturing."

U.S. Steel said it would remain American and grow bigger and stronger through a partnership with Nippon Steel, bringing massive investment, new technologies and thousands of jobs over the next four years.

A combination of the two companies would create the world's third largest steelmaker.

Without a deal, U.S. Steel has said it would step away from integrated steelmaking facilities and look at moving its headquarters from Pittsburgh.

Iron ore pellets produced at Minntac Mine and Keetac feed U.S. Steel's Mon Valley Works and Gary Works.

Minntac Mine is North America's largest taconite plant.

Nippon Steel has said it would bring technology to U.S. Steel facilities, abide by all existing labor agreements, not ship any jobs or production overseas, maintain pensions, profit-sharing, premium-free health care, keep U.S. Steel headquarters in Pittsburgh, and pay a \$5,000 bonus to hourly U.S. Steel employees and some others upon closure of a deal.

Stauber said he plans to attend Trump's Friday rally in Pittsburgh.



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGIONAL ADMINISTRATOR
REGION 5
77 WEST JACKSON BOULEVARD
CHICAGO, IL 60604-3590

Katrina Kessler, Commissioner
Minnesota Pollution Control Agency
520 Lafayette Road North
Saint Paul, Minnesota 55155-4194

Re: Minnesota's laws revising the Minnesota Pollution Control Agency's (MPCA) authority to regulate sulfate in NPDES permits

Dear Ms. Kessler:

I am writing regarding the U.S. Environmental Protection Agency's (EPA) review of two Minnesota session laws which, among other effects, impact MPCA's authority to include sulfate controls in National Pollutant Discharge Elimination System (NPDES) permits for discharges into wild rice production waters. Based on our review, we have determined that the session laws constitute a non-substantial change to Minnesota's NPDES program and are inconsistent with the Clean Water Act (CWA). EPA therefore disapproves the program revision affected by these laws as an improper modification to MPCA's authority to implement the NPDES program.

EPA has authority to review statutory or regulatory modifications of a state NPDES program under 40 C.F.R. § 123.62(a), which provides that EPA may initiate a program revision when necessary, including when the controlling state statutory or regulatory authority is modified or supplemented. Our review focused on two Minnesota laws¹: the "2015 Sulfate Law," Minn. Laws 2015, 1st Spec. Sess., Chapter 4, Article 4, Section 136 at (a)(1)(i)² [hereafter 2015 Sulfate Law]; and the "2016 Sulfate Effluent Compliance Law," Minn. Laws 2016, Chapter 165, Section 1 at (a) [hereafter 2016 Sulfate Law]. The details of our review are found in Enclosure A. EPA appreciates Minnesota's assistance with our review, including the Minnesota Attorney General's August 12, 2016 statement (*see* Enclosure B) and a February 11, 2021 meeting between EPA and MPCA.

As described in Enclosure A, our review found that the 2015 Sulfate Law and 2016 Sulfate Law: 1) limited MPCA's ability to include sulfate water quality-based effluent limits in NPDES permits that are required to comply with Minnesota's federally-approved sulfate water quality standard (WQS); and 2) invalidated sulfate effluent limits in any existing state permits,

¹ EPA did not review a third similar 2011 Minnesota law, Minn. Laws 2011, 1st Spec. Sess., Chapter 2, Article 4, Section 32 at (e), because that law's curtailment of sulfate controls in permits was limited to the "extent allowable under the Clean Water Act," and thus does not appear to have modified Minnesota's approved NPDES program.

² EPA's review and its findings in this letter are limited to the 2015 law's prohibition on permittee expenditures related to sulfate and do not extend to the remainder of the law which was not related to the Minnesota's approved CWA 402 program. We note that the 2015 Sulfate Law was amended by 2017 Minn. Laws Chapter 93, Article 2, Section 149 at (c) but not the clause reviewed by EPA.

respectively. Because the session laws both limit MPCA's authority to implement its approved NPDES program and improperly modify a facility's existing permit in contravention of the CWA, they constitute an improper modification to MPCA's authority to implement the NPDES program. 40 C.F.R. § 123.62(b)(3). As a result, the effluent limits and permit compliance schedules invalidated by the 2016 Sulfate Law remain subject to any federal enforcement and citizen action as provided for under the CWA. *See* 33 U.S.C. § 1319; 33 U.S.C. § 1365(a)(1).

Accordingly, EPA expects that MPCA's NPDES permits will include effluent limitations to meet all federally-approved WQS as required by the CWA, federal regulations, and EPA-approved Minnesota laws and rules. To this end, EPA urges MPCA to work with State lawmakers in resolving this matter.

Please contact me if you wish to discuss this matter, or your Assistant Commissioner for Water Policy and Agriculture can contact Tera Fong, Water Division Director, at fong.tera@epa.gov or (312) 886-6735.

Sincerely,

DEBRA
SHORE

Digitally signed by
DEBRA SHORE
Date: 2022.02.16
15:44:28 -06'00'

Debra Shore
Regional Administrator & Great Lakes National Program Manager

Enclosures

Enclosure A: A Review of Two Minnesota Sulfate Session Laws

The Clean Water Act (CWA) and federal regulations require states with approved National Pollutant Discharge Elimination System (NPDES) programs to maintain the authority needed to administer their programs in accordance with the CWA at all times. *See* 33 U.S.C. § 1342(c)(2); 40 C.F.R. § 123.63(a). Two Minnesota sulfate session laws -- Minn. Laws 2015, 1st Spec. Sess., Chapter 4, Article 4, Section 136 at (a)(1)(i) [hereafter 2015 Sulfate Law] and Minn. Laws 2016, Chapter 165, Section 1 at (a) [hereafter 2016 Sulfate Law] -- curtail the implementation of Minnesota's sulfate water quality standard (WQS) for wild rice producing waters in state issued NPDES permits. Because these two laws are not consistent with the CWA and its implementing regulations, they constitute an improper modification of Minnesota's approved NPDES program.

The CWA requires states to adopt WQSs subject to approval by the U.S. Environmental Protection Agency (EPA), which remain in effect unless and until EPA approves their modification. 33 U.S.C. § 1313(a) and (c); 40 C.F.R. § 131.21(a) and (e). Consistent with these authorities, Minnesota promulgated a sulfate WQS of 10 milligrams per liter (mg/L) applicable to waters utilized for the production of wild rice. *See* Minn. R. 7050.0224, subp. 2. Subsequently, EPA approved Minnesota's sulfate WQS. *See* 42 *Fed. Reg.* 56786, 56789 (Sept. 9, 1977). The CWA requires that NPDES permits include any requirements necessary to achieve the state's approved WQS. 33 U.S.C. § 1311(b)(1)(C); 40 C.F.R. § 122.44(d)(1) and (5), made applicable to state programs at 40 C.F.R. § 123.25(a)(15).

Minnesota's 2015 Sulfate Law and 2016 Sulfate Law revise the Minnesota Pollution Control Agency's (MPCA) authority to issue NPDES permits that are protective of Minnesota's federally approved sulfate WQS in at least two significant ways.

First, the 2015 Sulfate Law prevents MPCA from issuing permits that are protective of Minnesota's applicable federally approved sulfate WQS. This law provides in pertinent part, that "the agency shall not require [NPDES] permittees to expend money for design or implementation of sulfate treatment technologies or other forms of sulfate mitigation." Minn. Laws 2015, 1st Spec. Sess., Chapter 4, Article 4, Section 136 at (a)(1)(i). This law prevents MPCA from including effluent limits in NPDES permits that are needed to achieve Minnesota's federally approved sulfate WQS as required by 40 C.F.R. § 122.44(d)(1), on the basis that MPCA "has determined that the Wild Rice Standard is in need of substantial revision and therefore imprudent to apply."¹ Minnesota, however, has not revised the sulfate WQS and the sulfate WQS remains effective for CWA permitting purposes. Therefore, Minnesota's 2015 Sulfate Law is effectively a legislative limit upon MPCA's authority to issue NPDES permits that include effluent limits necessary to meet WQSs, 33 U.S.C. § 1311(b)(1)(C).

¹ *See* Letter from Lori Swanson, Minnesota Attorney General, to Tinka Hyde, Director, Water Division Region 5, August 12, 2016 [found at Enclosure B], at 4: "MPCA believes that it would be unreasonable for it to enforce the sulfate standard in existing permits because requiring compliance with the Standard would result in the expenditure of resources that may ultimately prove unnecessary. MPCA has advised EPA that it is 'pursuing options to reissue delayed mining NPDES permits quickly once there is a revised Wild Rice Standard [citation omitted]. . . . The above-described legislative restriction is strictly limited to the Wild Rice Standard, does not affect other water quality standards or MPCA's authority to enforce those standards, and is only in place until no later than January 15, 2018. In any event, as discussed above, MPCA has determined that the Wild Rice Standard is in need of substantial revision and therefore imprudent to apply."

Second, the 2016 Sulfate Law invalidates sulfate effluent limits in existing NPDES permits. In particular, the 2016 Sulfate Law provides that for NPDES permits meeting certain requirements:

- (1) the final sulfate limits resulting from implementation of the wild rice water quality standard in Minnesota Rules, part 7050.0224, subpart 2, are no longer valid; and
- (2) any compliance schedule permit conditions related to those final limits are no longer valid.

Minn. Laws 2016, Chapter 165, Section 1 at (a). According to the Minnesota Attorney General, the 2016 Sulfate Effluent Compliance Law aimed to remove effluent limits and a related schedule of compliance from the U.S. Steel Keetac facility.¹ Thus the 2016 Sulfate Law invalidated both the sulfate water quality-based effluent limits and compliance schedule related to those limits in contravention of the CWA and federal regulations. These include those federal requirements that NPDES permits include effluent limits necessary to achieve federally approved WQSs and incorporate schedules of compliance requirements where authorized under federal and state law. *See* 40 C.F.R. §§ 122.44(d)(1) and (5).² The 2016 law also circumvents federal regulations for modifying or revoking and reissuing permits (40 C.F.R. § 124.5(c)); public notice and comment procedures for permits (40 C.F.R. § 124.10); EPA's permit review (40 C.F.R. § 123.44); and the *Memorandum of Agreement between EPA and MPCA for the Approval of the State NPDES Permit Program*.³

Therefore, the 2016 Sulfate Law is a legislative limit upon MPCA's authority to issue NPDES permits that include effluent limits necessary to meet WQSs, 33 U.S.C. § 1311(b)(1)(C), and to implement permitting procedures consistent with the CWA, 33 U.S.C. § 1342(b)(3) and (4). Accordingly, the NPDES permit effluent limits and compliance schedules, invalidated by that law, remain subject to any federal enforcement and citizen action as provided for under the CWA. *See* 33 U.S.C. § 1319; 33 U.S.C. § 1365(a)(1).

¹ Letter from Lori Swanson, Attorney General, State of Minnesota, to Tinka Hyde, Division Director, U.S. EPA (August 12, 2016).

² 40 C.F.R. 124.5(c) (*Applicable to State programs, see 40 CFR 123.25 (NPDES), 145.11 (UIC), 233.26 (404), and 271.14 (RCRA)*).

(1) If the Director tentatively decides to modify or revoke and reissue a permit under [40 CFR 122.62](#) (NPDES), 144.39 (UIC), 233.14 (404), or 270.41 (other than § 270.41(b)(3)) or § 270.42(c) (RCRA), he or she shall prepare a draft permit under § 124.6 incorporating the proposed changes. The Director may request additional information and, in the case of a modified permit, may require the submission of an updated application. In the case of revoked and reissued permits, other than under [40 CFR 270.41\(b\)\(3\)](#), the Director shall require the submission of a new application. In the case of revoked and reissued permits under [40 CFR 270.41\(b\)\(3\)](#), the Director and the permittee shall comply with the appropriate requirements in [40 CFR part 124](#), subpart G for RCRA standardized permits.

(2) In a permit modification under this section, only those conditions to be modified shall be reopened when a new draft permit is prepared. All other aspects of the existing permit shall remain in effect for the duration of the unmodified permit. When a permit is revoked and reissued under this section, the entire permit is reopened just as if the permit had expired and was being reissued. During any revocation and reissuance proceeding the permittee shall comply with all conditions of the existing permit until a new final permit is reissued.

³ *See Memorandum of Agreement Between the United States Environmental Protection Agency and the Minnesota Pollution Control Agency for the Approval of the State NPDES Permit Program* (May 7, 1974), Section II, Agreement, Sections 124.44(d) (Schedule of Compliance in Issued NPDES Permits); and 124.72 (Modification, Suspension and Revocation of NPDES Permits).

Enclosure B: Minnesota Attorney General August 12, 2016 Letter



OFFICE OF THE ATTORNEY GENERAL

State of Minnesota

ST. PAUL, MN 55155

LORI SWANSON
ATTORNEY GENERAL

August 12, 2016

Tinka Hyde
Director, Water Division
United States Environmental Protection Agency
Regional Administrator
77 West Jackson Boulevard
Chicago, IL 60604-3590

VIA U.S. MAIL AND E-MAIL

Re: MPCA's Legal Authority to Implement its Authorized NPDES Program

Dear Director Hyde:

On April 5, 2016, you requested the Minnesota Pollution Control Agency ("MPCA") "provide an updated Attorney General's statement to explain whether the current scope of MPCA's authority remains adequate to issue permits in compliance with all applicable [Clean Water Act] requirements, including whether MPCA continues to have adequate authority to implement all of its federally approved water quality standards consistent with [Clean Water Act] Section 301(b)(1)(C)." You stated that legislation enacted in 2015 by the Minnesota Legislature "appears to modify and/or revise the authority of the State to administer its [National Pollutant Discharge Elimination System] program and implement its federally approved water quality standards." On June 28, 2016, you sent MPCA a second request to "provide an additional updated Attorney General's statement to explain whether the current scope of MPCA's authority remains adequate to enforce all conditions in those [National Pollutant Discharge Elimination System] permits to which the law is expected to apply." You reference legislation enacted by the Minnesota Legislature in 2016 and state it "appears to invalidate water quality based effluent limits and compliance schedules for sulfate that were included in certain [National Pollutant Discharge Elimination System] permits issued by the MPCA." You requested the Attorney General statements be provided by August 12, 2016.

I provide the following in response to both requests.

As you are aware, the Clean Water Act ("CWA") requires States to adopt water quality standards. See 33 U.S.C. § 1313(a). Pursuant to the CWA, Minnesota adopted such water quality standards in 1973, which are codified as Minn. R. ch. 7050. The U.S. Environmental

8-0552076

Tinka Hyde
Director, Water Division
United States Environmental Protection Agency
August 12, 2016
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Protection Agency ("EPA") approved these standards in 1977, including the sulfate standard applicable to "waters used for production of wild rice during periods when the rice may be susceptible to damage by high sulfate levels," which is codified as Minn. R. 7050.0224, subp. 2 ("Wild Rice Standard" or "Standard"). See 42 *Federal Register* 56786-03. Minnesota is the only State that has adopted a water quality standard for sulfate relating to waters containing wild rice. MPCA has expansive authority to issue and enforce National Pollutant Discharge Elimination System ("NPDES") permits. See Minn. Stat. chs. 115 and 116; Minn. R. chs. 7050, 7052 and 7053.

Under the CWA, "water quality standards should, wherever attainable, provide water quality for the protection and propagation of fish, shellfish and wildlife and for recreation in and on the water and take into consideration their use and value of public water supplies, propagation of fish, shellfish and wildlife, recreation in and on the water, and agriculture, industrial and other purposes including navigation." See 40 C.F.R. § 131.2. The CWA further directs that the "State shall from time to time, but at least once every three years, hold public hearings for the purpose of reviewing applicable water quality standards and, as appropriate, modifying and adopting standards." See 40 C.F.R. § 131.20. Once approved by the EPA, a State standard will continue in effect unless EPA approves a change. See 40 C.F.R. § 131.21(e). Neither the CWA nor its regulations, however, address what a State should do if it determines that an existing standard is in need of material revision and significant expenditures would unnecessarily be required of permittees to comply with the existing deficient standard.

The history of the Wild Rice Standard is described in *Minnesota Chamber of Commerce v. Minnesota Pollution Control Agency*, No. A12-0950, 2012 WL 6554544 (Minn. Ct. App. Dec. 17, 2012) ("MCC"). MPCA applied the Wild Rice Standard for the first time in 1975 to set a wastewater discharge sulfate limit for the Minnesota Power Clay Boswell steam power plant facility. *Id.* at 2. In 2009 and 2010, MPCA began taking steps to apply the Standard in the mining areas of northern Minnesota. *MCC* at 2. MPCA began a process to determine where the Wild Rice Standard should be enforced by asking certain mining companies to conduct surveys to detect the presence of wild rice in waters to which their facilities discharge wastewater to determine whether they are subject to the Wild Rice Standard for future permitting actions. *Id.* For the first time since 1975, in 2010 MPCA relied upon the Wild Rice Standard to set a discharge limitation in a permit for U.S. Steel. *Id.* MPCA's interpretation of the Wild Rice Standard as applicable to protect natural (uncultivated) wild rice was challenged in the *MCC* case. *Id.* At around the same time, related concerns were raised by MPCA and others regarding the accuracy of the science behind the Standard and how to define "waters used for production of wild rice." See <https://www.pca.state.mn.us/sites/default/files/wq-s6-40b.pdf>.

In response to these concerns, the Minnesota Legislature enacted legislation appropriating money and requiring MPCA to "adopt and implement a wild rice research plan using the money appropriated to contract with appropriate scientific experts." Minn. Laws 2011, 1st Spec. Sess., Chapter 2, Article 4, Section 32 at (d) ("2011 Wild Rice Legislation"). This

Tinka Hyde
Director, Water Division
United States Environmental Protection Agency
August 12, 2016
Page 3

legislation temporarily restricted MPCA from requiring permittees "to expend funds for design and implementation of sulfate treatment technologies," but provided for an exception if necessary to comply with "federal law." *Id.* The 2011 Wild Rice Legislation further provided that "nothing shall prevent the [MPCA] from including in a schedule of compliance a requirement to monitor sulfate concentrations in discharges and, if appropriate, based on site-specific conditions, a requirement to implement a sulfate minimization plan to avoid or minimize sulfate concentrations during periods when wild rice may be susceptible to damage." *Id.* Based in part on the 2011 legislation, the *MCC* decision concluded that the issues raised in the case were moot. *MCC* at 9.

After receiving the funding from the 2011 Wild Rice Legislation, the MPCA contracted with the University of Minnesota to research how sulfate affects wild rice. See <https://www.pca.state.mn.us/water/wild-rice-sulfate-standard-study>. The research was based on a scientific protocol developed in 2010-2011 by the MPCA in consultation with your agency, Minnesota Tribes, and the Minnesota Department of Natural Resources as well as input from interested and affected stakeholders. *Id.*

In 2014, MPCA published the initial results of its research, which demonstrated that sulfate is not directly toxic to wild rice, but it can be converted into sulfide, which is toxic. The research supported material revisions to the Standard. See "Wild Rice Sulfate Standard Study Preliminary Analysis" at <https://www.pca.state.mn.us/sites/default/files/wq-s6-42w.pdf>; See also "Wild rice study reveals more questions for state officials," at <https://www.nprnews.org/story/2014/03/12/wild-rice-study>. The study found that the presence of iron in the water may mitigate the conversion of sulfate to sulfide. The research also found instances where higher levels of sulfate in waters did not result in high levels of the toxic sulfide. See <https://www.pca.state.mn.us/sites/default/files/wqetc>. The Study determined that site-specific standards may be needed for some waters particularly where sulfate is more efficiently converted to sulfide and/or sediment iron levels are not sufficient to mitigate sulfide concentrations. *Id.*

In December 2014, MPCA sought feedback on a preliminary proposed permit that required U.S. Steel to comply with the existing Standard at its "Minntac" facility. See <https://timberjay.com/stories/us-steel-fighting-strict-pollution-rules/11955>.

In the 2015 legislative session, Minn. Laws 2015, 1st Spec. Sess., Chapter 4, Article 4, Section 136 ("2015 Wild Rice Legislation") was enacted. The 2015 Wild Rice Legislation prohibits MPCA from taking any actions to implement the standard that would require a permittee "to expend money for design or implementation of sulfate treatment technologies or other forms of sulfate mitigation." *Id.* at (a)(1). The legislation did not provide an exception for enforcement necessary to comply with federal law as the 2011 legislation did, but the 2011 Wild Rice Legislation was not repealed. *Id.* The 2015 legislation requires MPCA to complete rulemaking to promulgate a new Wild Rice standard by January 15, 2018. *Id.* at (c). In 2016, the Legislature enacted a statute that essentially applied the substance of the 2015 legislation to

Tinka Hyde
Director, Water Division
United States Environmental Protection Agency
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the U.S. Steel Keetac facility, which was subject to a "schedule of compliance" requiring it to take actions necessary to comply with the Standard. Minn. Laws 2016, Ch. 165, sec. 1. The legislation does not apply or restrict the issuance, reissuance or enforcement of any future permits. MPCA has advised this Office that the Keetac facility is idled.

Minnesota Governor Mark Dayton signed the 2011, 2015 and 2016 legislation relating to the revision of the Wild Rice Standard. He publically stated that the Standard is "antiquated" and "not even based on current science directly related to the conditions we're trying to deal with." See <http://www.mprnews.org/story/2015/03/25/mpca-wild-rice>. MPCA similarly has stated that "using the same limit for every river or lake where wild rice grows doesn't make sense, because many factors influence whether wild rice will thrive." See <http://www.mprnews.org/story/2015/03/25/mpca-wild-rice>. MPCA has also stated that "[n]o single sulfate value would be protective of wild rice at all sites." Based on its research, MPCA is developing an equation to calculate appropriate values on a site-by-site basis, reflecting its expert conclusion that higher levels of iron can lead to less sulfide, and higher levels of organic carbon can lead to more sulfide. See <https://www.pca.state.mn.us/sites/default/files/wq-s6-43k.pdf>. In March of 2015, MPCA "announced that it wants to eliminate the current standard of 10 milligrams of sulfate per liter of water in wild rice waters, and begin to apply different standards to different waters based on their chemical makeup." See <https://www.minnpost.com/environment/2015/05/despite-pressure-lower-mnntac-sulfate-emissions-status-quo-could-last-awhile>.

MPCA believes that it has made significant progress towards completion of the scientific studies regarding a new Wild Rice Standard and states it will commence the formal rulemaking process soon. See <https://www.pca.state.mn.us/sites/default/files/wq-rule4-15a.pdf>. The CWA provides States with the authority to revise water quality standards. 40 C.F.R. § 131.20. MPCA, on behalf of the State of Minnesota, is exercising this authority regarding the Wild Rice Standard, based on its scientific evidence indicating that the existing standard requires substantial revision. MPCA believes it would be unreasonable for it to enforce the sulfate standard in existing permits because requiring compliance with the Standard would result in the expenditure of resources that may ultimately prove unnecessary. MPCA has advised EPA that it is "pursuing options to reissue delayed mining NPDES permits quickly once there is a revised Wild Rice Standard." See July 13, 2016 letter from MPCA to EPA. MPCA has apprised this Office that it is "evaluating each site to determine what data could be gathered during this interim period to ensure [it] ha[s] all data necessary to move expeditiously on reissuances once there is a revised wild rice standard."

The above-described legislative restriction is strictly limited to the Wild Rice Standard, does not affect other water quality standards or MPCA's authority to enforce those standards, and is only in place until no later than January 15, 2018. In any event, as discussed above, MPCA has determined that the Wild Rice Standard is in need of substantial revision and therefore imprudent to apply.

Tinka Hyde
Director, Water Division
United States Environmental Protection Agency
August 12, 2016
Page 5

MPCA also believes that it has adequate authority to revise the applicable Standard, and once the Standard is revised (subject to EPA approval), it will have full and unrestricted authority to enforce the Standard. Accordingly, under these unique circumstances, and in accordance with MPCA's expert opinion regarding the deficiencies of the current Standard, I agree with MPCA that it has adequate authority to implement federal law by revising the current Standard that MPCA believes is deficient and then enforcing it as modified.

This analysis is supported by court decisions holding that the CWA is to be given a reasonable interpretation which is not parsed and dissected with the meticulous technicality applied in testing other statutes and instruments. *See Envtl. Def. Fund, Inc. v. Costle*, 657 F.2d 275, 292 (D.C. Cir. 1981). The EPA has the ability to be flexible with regard to revision of State water quality standards, and has exercised this discretion in other cases.

Indeed, if a State fails to fix a deficient water quality standard, 40 C.F.R. § 131.22 requires EPA to replace it with a federal standard in 90 days, but EPA's decision to delay replacement was upheld when EPA reasonably provided Montana more time to modify a deficient standard. *See American Wildlands v. Browner*, 94 F. Supp. 2d 1550, 1165 (D. Colorado 2000) (Montana's stated intention given weight in decision upholding EPA's decision to allow Montana extra time to submit an amended water quality standard). Minnesota's legislation does not allow substantially more time for MPCA to revise the Wild Rice Standard than was allowed for Montana to complete its revision. *Id.* The D.C. Circuit has also recognized that sometimes "it is logical that EPA should refrain from acting until the states have completed an initial effort to update the standards as they deem appropriate." *See Envtl. Def. Fund, Inc. v. Costle*, 657 F.2d 275, 294 (D.C. Cir. 1981) ("EPA's task of determining the need for revised or new salinity standards to meet the Act's requirements would be greatly simplified by its temporary deference.")

Based on the above facts and circumstances of this matter and legal analysis, including the representations of MPCA and the deference owed to its expertise regarding the subject Standard, it is my opinion that the laws of the State of Minnesota provide adequate authority for MPCA to carry out the program elements in 33 U.S.C. § 1342 pertaining to water quality standards.

Sincerely,



LORI SWANSON
Attorney General

Dan Kraker · Duluth, Minn. · November 30, 2018 7:02 PM

State officials issue permit for Minntac tailings basin



U.S. Steel's Minntac taconite mine and plant looms over the city of Virginia on April 10, 2015. Dan Kraker | MPR News

Save 

SHARE

State officials plan to issue a water quality permit Saturday to U.S. Steel for a massive mining waste pit on the Iron Range.

ON AIR

A CONVERSATION WITH NEW YORK TIMES COLUMNIST DAVID FRENCH WITH KERRI MILLER

MCEA Exhibit 9

The water pollution discharge permit from the Minnesota Pollution Control Agency lays out a timeline for the company to update a tailings basin that covers more than 13 square miles at Minntac, the state's largest taconite mine, in Mountain Iron.

The basin, which contains a mixture of water and leftover mine processing waste, has been in use since 1967, before modern environmental laws were in place. For many years, high concentrations of pollutants, notably sulfate, have seeped from the basin into nearby waters.

U.S. Steel has operated the basin with an expired permit since 1992.

▼ **February:** [U.S. Steel, state settle dueling lawsuits over Minntac water permit](#)

Tribes and environmental groups have pushed the state for years to issue a new permit for the facility. The MPCA acknowledges that the tailings basin has long discharged several pollutants into surrounding waters at levels that exceed state and federal standards.

Of most concern is sulfate, which at high levels can inhibit the growth of wild rice. Tribal groups say wild rice in the area that they harvested historically has disappeared.

The MPCA issued a draft permit in November 2016, less than a week after environmentalists had sued over the state's oversight of the mine.

"So it's been a challenging permit to reissue, and it's taken us a lot of time, but we're very pleased to be issuing a permit that has a path for meeting surface and groundwater standards and a timeframe for achieving that," said Shannon Lotthammer, assistant commissioner for water policy at the MPCA.

Lotthammer said the permit includes requirements that are designed to meet surface water and groundwater standards, but that it does so in a way that also allows for the ongoing operation of Minntac.

The taconite mine and processing plant employ around 1,400 people on the Iron Range, and U.S. Steel officials had warned that excessive regulations could threaten jobs at the facility.

"U.S. Steel is pleased that MPCA acted to reissue the administratively-rended Minntac Tailings Basin NPDES permit. We are currently reviewing permit and evaluating the conditions." U.S. Steel spokesperson Meghan Cox said in a statement.

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But Paula Maccabee, attorney with the environmental group WaterLegacy, blasted the permit, saying it lets U.S. Steel off easy and doesn't do enough to address serious water pollution concerns at the site.

She also said the federal Environmental Protection Agency failed in its oversight of the Clean Water Act.

"For years EPA has said, 'State of Minnesota, you've got to have permits that control sulfate and protect your fish and protect your wild rice,' and here's the biggest, most important permit, and the EPA walked away and didn't do their job," she said.

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