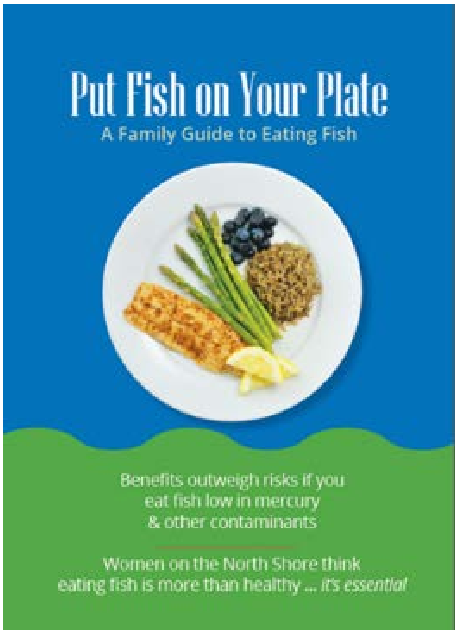
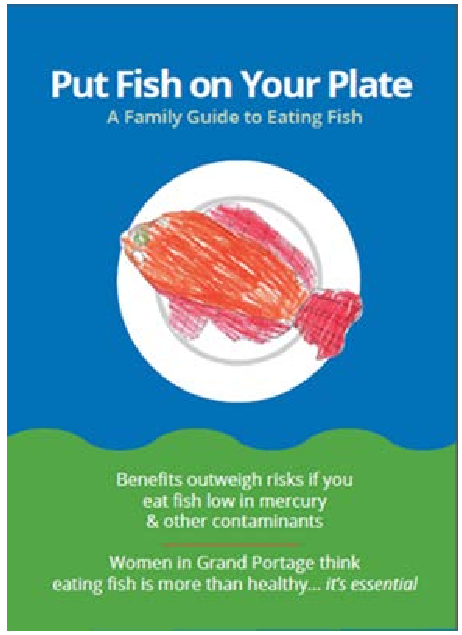
Technical Report

Fish are Important for Superior Health (FISH) Project





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Most of all, we are indebted to the 499 women who shared their time and stories for the FISH Project. You made this project a success.

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Summary

A 2011 study by MDH showed that 10% of newborns tested in the North Shore region had mercury above levels of concern in their blood. Too much mercury can cause lasting problems with understanding and learning. The 2011 study results spurred a collaboration in 2013 among Sawtooth Mountain Clinic (SMC), Grand Portage Health Service (GPHS), North Shore Health (NSH), Grand Portage Band of Lake Superior Chippewa Trust Lands, and the Minnesota Department of Health (MDH), resulting in the Fish are Important for Superior Health (FISH) Project. The FISH Project's goals were to reduce mercury exposure in women in the area and pilot an in-clinic screening for high mercury exposure. Nearly 500 women from Cook County, Grand Portage, and the surrounding area participated in the FISH Project.

FISH participants provided information about which fish they ate and how often they ate fish. They received information about healthy diets that included which type (species) of fish to eat and how often they can eat fish. They also had a blood sample analyzed for mercury and healthy fatty acids. Now that the project is complete, all blood samples have been destroyed.

Compared to women in a national study, women in the FISH Project:

- reported eating more fish; and
- had higher fatty acids levels and blood mercury levels. However, only 3% of women (about the same percentage as the national study) had mercury levels above the level of concern (5.8 micrograms per liter).

Mercury levels were lowest in blood collected in the spring and highest in fall samples. Results from FISH support the findings of the 2011 study.

An important finding from the project is that women did not stop eating fish as a result of the participating in the FISH Project. Studies have shown that fish can provide important nutrients that help fetuses and babies develop, as long as they are low in mercury and other contaminants. The fatty acids, vitamins, and minerals in fish are also important for adult health.

Some of the women in the FISH Project participated in a follow-up clinic visit six months after their initial visit. Changes at the follow-up were positive:

- Participating in the project did not cause women to eat less low-mercury fish; many women said they ate more fish since their initial visit.
- Fatty acid levels did not change.
- Mercury levels were lower at the follow up visit.
- Participants with elevated mercury at the initial visit reduced their consumption of fish species shown to contribute most to higher mercury exposure, such as walleye and lake trout.

On-going education will be incorporated into local efforts as a result of the FISH Project.

- SMC and GPHS clinics will include screening for high mercury in future prenatal visits. (In the FISH Project, using a brief group of questions about fish consumption was shown to be a useful tool to identify women with potential high mercury exposure and those who might benefit from increased fish consumption.)

- Community education and WIC visits will include information about choosing which fish to eat and how often. Learning opportunities may include local events (such as the Grand Portage Health Fair in June) and a mailing to all women ages 18-50 who use GPHS.

Based on findings from the FISH Project and other research, a new brochure and website following MDH's fish consumption guidelines were developed by HealthPartners Institute to reach more women and families with clear, easy-to-understand information. The website **Chooseyourfish.org** helps people navigate the many fish choices and choose the best ones for women who might become pregnant or who are already pregnant. It also provides tips on how to select and cook fish, including recipes and short videos. Versions of the brochures were designed for the North Shore and Grand Portage communities and will be distributed by FISH Project partners.

Project Background/Introduction

Fish and fishing are strong parts of the culture and history of the communities in Minnesota along the North Shore of Lake Superior. Most human exposure to methylmercury (meHg) is from consumption of fish. The developing nervous system is especially vulnerable to negative developmental impacts from exposure to meHg. However, benefits of fish consumption, including improved eye and brain development, outweigh risks if fish that are low in contaminants are eaten. Thus, consumption of fish low in mercury (Hg) and other contaminants should be encouraged in women of childbearing age.

MDH began targeting women of childbearing age (WCBA) with fish consumption guidelines related health education in the mid-1990s. A study from 2001 reported 25% of women from MN who participated in the survey were aware of fish consumption advice (Anderson et al. 2004). Women from Minnesota surveyed as part of a more recent study (Connelly et al. 2013) reported an overall awareness of 80 to 88 percent and 37 to 46 percent awareness before pregnancy. A survey of Minnesota women who recently gave birth showed that if a woman remembered receiving a Minnesota Department of Health (MDH) fish consumption brochure, she reported receiving it from a health care provider (Scher et al. 2012).

Health care providers are a trusted source of information to their patients and could serve an important role in preventing meHg exposure in babies (Gliori et al. 2006; Teisl 2011). Providers do not routinely screen WCBA or pregnant women for Hg exposure. Health care providers may not be well trained to test, interpret test results, or provide guidance about the risk of elevated meHg exposure versus the benefits of fish consumption. Testing blood is a direct measure for identifying elevated meHg exposure and determining risk among those frequently consuming fish. However, some health care providers may use urine mercury to screen for excessive mercury exposure, although blood mercury levels are a better measure of meHg exposure. Adding questions about fish consumption habits to the electronic medical record (EMR) intake form for WCBA could be used to screen for elevated Hg exposure and indicate who should receive an intervention of counseling about fish consumption choices. Screening questions could be used alone or in combination with testing blood. Through an analysis of the National Health and Nutrition Examination Survey (NHANES) data, Buchanan et al. (2015) found that if health care providers were to begin testing for mercury exposure, pre-screening with questions about fish consumption could reduce the number of mercury tests needed by 50%.

A recent study found that 10% of newborns tested in the Lake Superior basin region of Minnesota had blood mercury levels above the level equivalent to the U.S. EPA reference dose (RfD) for methylmercury

(5.8 µg/L) (McCann 2011). The Sawtooth Mountain Clinic (SMC), Grand Portage Health Service (GPHS), North Shore Health, and Trust Lands Grand Portage Band of Lake Superior Chippewa (GPC) (located on the North Shore of Lake Superior) partnered with the MDH to implement screening questions in the EMR to identify WCBA with elevated mercury and who need advice about choosing low mercury fish. The Fish are Important for Superior Health (FISH) Project was designed to intervene with WCBA to: reduce Hg exposure in a community where elevated exposure in infants has been shown, evaluate screening questions for predicting blood Hg levels, and optimize screening questions to provide greatest utility across populations. Women were asked three simple screening questions to determine if this easily implemented screen would identify women with high mercury in their blood. Women also completed a more detailed questionnaire about fish consumption and had their blood tested for total mercury. Women who participated in the FISH Project learned about the risks and benefits of eating fish and how to enjoy the health benefits of fish consumption while reducing their exposure to mercury.

The FISH Project took place in an engaged community where there is a documented need to reduce mercury exposure. WCBA from the Grand Marais and Grand Portage communities expressed interest in learning more about risks and benefits of fish consumption and have reported frequently eating fish species with one meal per month fish consumption advice (Lauber et al. 2011). Results from *Mercury in Newborns* showed a seasonal exposure pattern with the highest exposures occurring in babies born in summer months. This pattern suggests local fish consumption is an important source of mercury exposure in this community. This project worked to reduce mercury exposures in WCBA by incorporating an intervention into clinical practice at SMC and GPHS, clinics located on the North Shore of Lake Superior in the Grand Marais and Grand Portage communities. Fish consumption advisories provide general guidance rather than individual advice. By training local health care providers and providing an opportunity for blood tests, appropriate individual health advice was delivered.

As a Federally Qualified Health Center and the only safety net, primary health care clinic in the area, the SMC staff strives to improve the care of patients and the health of the surrounding communities. This project was an opportunity to improve health. SMC's board of directors, clinical staff, and its patients agreed to do whatever was needed to educate women who are or may become pregnant and ultimately reduce mercury exposure in future babies. Collaboration between MDH researchers, SMC and GPHS health care providers, and community members created vital and lasting relationships. The commitment of existing and new patients who chose to participate in this study, *together*, created a rich and robust learning environment and shared the knowledge that women can eat fish and give birth to healthy babies.

The Grand Portage community is located entirely within one mile of Lake Superior, with most residents living on or very near the shore. Adverse health effects as well as health benefits of fish consumption are a concern to many Grand Portage residents. There is no human development, other than roads and trails around any of the reservation's inland waters. There are no industrial or commercial sources of mercury on the reservation; yet, the fish are polluted. Subsistence netting is a traditional cultural practice in Lake Superior. Grand Portage has been measuring fish mercury levels periodically since 1992; elevated levels have been found in several species of fish used for subsistence by the Band, including lake cisco, lake trout, walleye, and northern pike. Mercury in the fish tissue consumed by tribal members is not attributable to sources under the Tribe's jurisdiction. The elevated mercury concentrations found in fish are due primarily to atmospherically deposited mercury bioaccumulating in the food chain from sources outside the Reservation. Grand Portage Band of Lake Superior Chippewa

Trust Lands actively work to eliminate mercury pollution in the waste-stream but does not have the ability to regulate mercury in the air or beyond the boundaries of the Reservation and its waters.

Methods

Brief summaries of methods are provided below. Detailed procedures and quality assurance are provided in Appendix A (Protocol) and B (QAPP).

Health Care Provider Training

Training and resources about risks and benefits of eating fish and the importance of reducing exposures in women of childbearing age were developed for and evaluated by FISH Partner providers and nurses. Trainings included courses developed by University of Illinois at Chicago and Michigan State University as well as a course developed by Stony Brook (Silbernagel 2011). The training courses were evaluated by MDH for consistency with Minnesota and Great Lakes Consortium fish consumption advisories, and by SMC/GPHS providers and nurses regarding feasibility for use by providers and nurses in a clinical setting (see Health Care Provider Training Report in Appendix C).

Educational Brochure

Community-specific communication materials were developed to support fish consumption choices that promote the benefits of eating fish while minimizing exposures to contaminants in fish. Materials were developed with input from SMC and GPHS and results from other research (Connelly et al. 2014; Lauber et al. 2011; Niederdeppe et al. 2015). (Brochures are in Appendix D.)

Participants

Between June 2014 and July 2015, volunteer women aged 16 to 50 were enrolled in the FISH Project. To be eligible, women needed to meet the following criteria: current or potential client of Sawtooth Mountain Clinic (SMC) or Grand Portage Health Service (GPHS); permanent resident in Cook County, MN, or the surrounding area; willing to provide a blood sample; and willing to complete a follow-up clinic appointment six months after their initial clinic appointment, if requested. Women were recruited by clinic nurses, posters displayed at community locations and events, advertisements in local newspapers, public service announcements on local radio, and news stories by local media (see Promotion Summary in Appendix E). During the three years prior to the start of enrollment, 793 women age 16 to 50 had been seen at one of the clinics; 499 women were enrolled and completed a clinic visit. All participants provided written informed consent. The Minnesota Department of Health Institutional Review Board and the US EPA Human Subjects Research Review Official approved all study protocols.

Clinic Visit

At the clinic visit conducted at SMC, GPHS, or a temporary clinic, a nurse obtained written informed consent, administered three mercury screening questions from the Electronic Medical Record (EMR) and a paper questionnaire to collect detailed information on fish consumption, provided education on fish consumption choices, and distributed incentives. Venous blood was drawn by venipuncture and stored in Vacutainers by NSH or GPHS staff. Samples collected by GPHS or at a temporary site were refrigerated up to 24 hours and transferred to NSH. Samples were stored at -20ºC or below at NSH, shipped overnight with ice packs, and stored at -20ºC or below until analysis at MDH Public Health Laboratory. Blood samples were destroyed by autoclave after analysis. (See Appendix F: Sample Disposal Documentation.)

Clinic appointment status and responses to the mercury screening questions were recorded and tracked through Centricity Electronic Health Record/Practice Management. Participants were assigned a unique project identification number. All data transferred to MDH was de-identified. Responses to the mercury screening questions were provided to MDH electronically. Completed detailed questionnaires were entered into an ACCESS database by MDH.

Women with blood mercury levels above the EPA RfD, along with two time-paired participants for each participant with elevated mercury, completed a follow-up visit identical to their initial clinic visit six months after their initial visit.

Biomonitoring
MDH Public Health Laboratory (PHL) analyzed blood samples for mercury and fatty acids. Results were reported to participants through letters from the clinics. Fatty acids were analyzed in conjunction with mercury to enable assessment of our objective to ensure that women of childbearing age have access to information that will help them take action to reduce exposures to mercury while gaining the benefits of fish consumption.

Total mercury was analyzed in whole blood from all 499 participants using the inductively coupled plasma mass spectrometry (ICP-MS) method from the Centers for Disease Control and Prevention (CDC), Blood Metals Panel 3 (BMP3) ICP-DRC-MS. Blood samples with total mercury greater than the blood level equivalent to the EPA RfD (5.8 µg/L) were reanalyzed by Brooks Rands Labs, LLC to speciate mercury. Speciation allowed verification of fish consumption as the source of exposure.

Omega-3 fatty acids in fish have been associated with healthy fetal neurodevelopment and are thought to be beneficial for brain and eye development in the fetus. The analysis of both saturated and unsaturated fatty acids in blood plasma from 490 participants was performed by derivatization into methyl esters and liquid/liquid extraction followed by gas chromatography mass spectrometry (GC/MS) analysis. Plasma samples from nine participants could not be analyzed due to insufficient sample volume or a damaged vial.

Fish Consumption
Participants reported their fish consumption by two methods. All 499 women (age 16-50) answered brief screening questions and completed a detailed questionnaire. Responses from the two methods were compared.

Mercury Screening Questions (EMR Screening Questions)
The screening questions (Table 1) were designed as a screen that could be quickly done in a clinic setting to predict elevated mercury exposure. The questions asked about fish participants had eaten in the last 2-3 months. This time period was selected to correspond to the 50-70 day half-life of methylmercury in blood and provide a long enough time period to allow reporting of species eaten infrequently. Grouping species by mercury concentration was a factor considered in the design of the screening questions. Responses to the screening questions were compared to blood mercury measured in participants to assess whether the questions were a good predictor of mercury exposure.

The first question asked about overall fish consumption. The second question asked about consumption of local fish species with moderate levels of mercury warranting advice to limit consumption to one meal per month. Consumption of fish with moderate levels of mercury is likely a better predictor of elevated mercury exposure than overall fish consumption, but assessment is not as simple as overall fish consumption because popular species of fish with moderate mercury vary among communities. Species included in this question were those project partners and focus group responses indicated were being consumed in these communities (Lauber et al. 2011). Initially, the third question asked whether the participant ate shark or swordfish. So few women reported eating these species (2/351) that the third question was changed in January 2015 to ask about consumption of canned tuna. One hundred forty-eight participants answered the revised question about canned tuna.

Table 1. EMR Screening Questions

EMR Screening Question	Comments
1. In the last 2-3 months, how many times a week did you eat any kind of fish?	All fish, not selective for mercury level. Broadly applicable across populations.
2. In the last 2-3 months, how many times a month did you eat any of these fish – Walleye, Northern Pike, Bass, or Lake Trout from Lake Superior?	"Select moderate mercury fish" Very specific to the community: • Species included were those that are moderately high in mercury (one meal per month advice, Hg concentrations in the range of 0.23 – 1 ppm) • Information from focus groups and FISH Project partners suggested these species were eaten frequently by the community
3. In the last 2-3 months, did you eat shark or swordfish?	Species high in mercury, greater than 1 ppm. Species chosen based on local availability in the market. So few women reported eating these species that question 3 was revised.
3. (revised) In the last 2-3 months, how many times per month have you eaten canned tuna?	Canned tuna had the highest mean consumption per week by participants. Canned tuna can have low (canned light) or moderate (canned white/albacore) levels of mercury.

Detailed Questionnaire (DQ)

Questions on age, education, omega-3 supplements, fish serving size (the number of palm-sized pieces of fish eaten in a meal), and which species of fish the participant had eaten in the past year and past week were included in the first part of the DQ. For each species eaten in the past year, the participant then completed details about consumption frequency of that species by season and source over the past year. Given the seasons in Minnesota, frequency of consumption of locally-caught fish can vary quite a bit by season. Again, the species list was based on responses in focus groups and project partner input. Participants could write-in species not listed in the questionnaire. (See Table 2.)

Table 2. Species Listed in Detailed Questionnaire

Species Listed in Detailed Questionnaire	Hg Grouping	Hg Concentration in Fish (ppm)	Consumption Advice
Cod, Fish sticks, salmon, shellfish, tilapia	Very low Hg	<0.11	2 meals per week
Canned light tuna, panfish, perch, lake herring, stream trout whitefish	Low Hg	0.11 – 0.22	1 meal per week
Bass, halibut, lake trout, northern pike, tuna (canned white, steak/fillet), walleye	Moderate Hg	>0.22 – 0.95	1 meal per month
Swordfish, shark	High Hg	>0.95	Do not eat

Fish consumption reported in the DQ for the three months prior to the clinic appointment and in the past week were used to construct responses to the EMR screening questions. Additional groupings of species (Fish Metrics – Table 3) were calculated to explore improvements in the screening questions: 1) total fish intake, excluding shellfish; 2) total fish, excluding low mercury fish (shellfish, salmon, fish sticks/sandwiches, tilapia); and 3) all moderate mercury fish intake (walleye, northern pike, bass, lake trout, halibut, and tuna steak/fillet). All consumption measures were converted to meals in past week to allow comparisons of associations of fish intake with blood Hg.

Table 3. Fish Metrics

EMR Screening Questions
Fish and shellfish meals
Select moderate Hg fish meals[1]
Canned tuna fish meals (n=148)
Detailed fish consumption in past 3 months
Fish and shellfish meals
Select moderate Hg fish meals[1]
Canned tuna fish meals
Fish meals, excluding shellfish
Fish meals, excluding low Hg meals[2]
All moderate Hg fish meals[3]
Detailed fish consumption in past week
Fish and shellfish meals
Select moderate Hg fish meals[1]
Canned tuna fish meals
Fish meals, excluding shellfish
Fish meals, excluding low Hg meals[2]
All moderate Hg fish meals[3]

[1]Select moderate Hg fish = walleye, northern pike, bass, and Lake Superior lake trout.
[2]Low Hg fish = shellfish, tilapia, salmon, and fish sticks.
[3]All moderate Hg fish = walleye, northern pike, bass, lake trout, halibut, and tuna (steak/fillet).

Statistical Analysis

Bivariate associations of fish, fatty acids, and blood mercury with participant characteristics were examined using ANOVA tests, while Fisher's exact tests were used to evaluate associations of participant characteristics with blood mercury greater than 5.8 µg/L (which is the blood concentration equivalent to EPA's Reference Dose (RfD) for meHg of 0.1 µg/kg/day).

Associations of intake of individual fish species and fish consumption metrics with log-transformed DHA, EPA, DHA&EPA, and blood mercury, and with blood mercury greater than 5.8 µg/L were analyzed using simple linear and logistic regression, respectively. Individual fish and fish metrics that best predicted fatty acids, blood mercury, or elevated blood mercury were identified using stepwise selection in multivariable models that adjust for age, education, usual number of fish piecess/meal, and sampling season. Fatty acid models also adjusted for use of omega-3 fatty acid supplements and supplemented foods. Multiple linear regression models were fitted with the natural log-transformed Hg, DHA, EPA, or DHA&EPA concentrations on the predictor variables and are presented as exponentiated model coefficients, which can be interpreted as the proportional change in the geometric mean associated with an increase in 1 fish meal/week with adjustment for other predictors in the model.

Wilcoxon signed rank tests for paired data were used to examine changes in mercury, fish consumption, and fatty acids at baseline and 6 months in the 45 participants with follow up, overall, and stratified by group (blood Hg >5.8 µg/L versus ≤ 5.8 µg/L at baseline). Repeated measures models were constructed to examine across group differences in fish consumption and blood mercury at baseline and 6 months (SAS PROC MIXED). Models included a group variable (blood Hg >5.8 µg/L versus ≤ 5.8 µg/L at baseline), time variable (baseline versus 6 months), and the interaction of time and group, which assessed significance of across-group changes in mercury or fish consumption at the 6 month visit. Based on significant differences in fish consumption and blood mercury by sampling season in bivariate analyses, repeated measures models also adjusted for season of assessment.

Two-sided Wilcoxon Mann Whitney tests were used to compare blood mercury levels and responses to EMR Screening Questions from the FISH Project and Mercury Screening Project. Blood mercury, fish consumption, and plasma fatty acids from the FISH Project were also compared with data from the National Health and Nutrition Examination Survey (NHANES), which is a representative sample of the non-institutionalized U.S. population. Summary measures (median, mean or geometric mean, and proportion with blood Hg >5.8 µg/L) were estimated for female NHANES participants 16-50 years of age using survey sampling design variables. Summary measures for FISH participants were compared with NHANES summary measures using one-sample t-tests for means and a binomial test for the proportion with blood Hg >5.8 µg/L.

The utility of fish metrics as a screening test predicting Hg was explored considering a value of Hg ≥ 5.8 µg/L as the "gold standard." Receiver operator characteristic (ROC) curves for each fish metric were created by plotting the calculated sensitivity against 1-specificity using SAS. Finally, sensitivity and specificity were examined for individual fish metrics and for combinations of fish metrics considered simultaneously (in other words, as parallel screening tests). For parallel screening, net sensitivity was calculated using those identified as positive by either test, and net specificity was calculated using those identified as negative by both tests (Gordis 2009). Using parallel screening tests results in increased sensitivity and negative predictive value. Positive predictive values and negative predictive values were calculated for the various screening tests using observed prevalence of elevated Hg in the entire population.

Results

Fish Consumption

Summaries of responses to the EMR Screening Questions and DQ are in Appendix G. These results were reported to the community at events in Grand Marais and Grand Portage in November 2015 (see Community Events Report in Appendix H).

Mean and maximum number of shellfish and finfish meals in the 3 months prior to clinic appointment calculated from responses to the DQ are shown in Table 4. Ninety-six percent of participants reported eating fish.

Table 4. Individual Fish Species Consumed in Past 3 Months (n=499)

Fish Species	Mean Number of Meals	Maximum Number of Meals
Tuna, Canned	4.20	54
Shellfish	2.99	91
Salmon	2.57	65
Lake Trout	2.31	30
Walleye	2.26	39
Lake Herring	2.13	64
Whitefish, Menominee	1.50	37
Fish Sticks/Sandwiches	1.01	26
Tuna Steak	0.74	33
Cod	0.59	13
Tilapia	0.48	21
Stream Trout	0.47	26
Other Fish	0.44	39
Northern Pike	0.42	26
Perch	0.24	38
Bass	0.15	9
Panfish	0.17	13
Halibut	0.08	6

Fish consumption reported in the DQ is on average 3 times higher than reported through the EMR Screening Questions. Other studies have also indicated that reported fish consumption increases with increasing number of survey questions on fish consumption (Oken et al. 2013). In addition to the number of questions, differences in reported fish consumption could also be influenced by: 1) timing - the DQ was administered later in the clinic appointment than the EMR Screening Questions giving more time for the participant for recall; and 2) visual versus verbal communication - the EMR screening questions were read to the participant whereas the participant completed a paper form of the DQ.

Associations of participant characteristics with fish consumption metrics were consistent across the fish metrics, except for canned tuna fish meals (Table 5). Fish consumption rates were greater in women who consumed larger meals and in the summer and fall seasons.

Table 5. Associations of Participant Characteristics with Fish Metrics in Last 3 Months

		DQ-calculated EMR Screening Questions			Additional DQ-calculated Fish Metrics		
Characteristic	**N**	**Mean Fish & Shellfish Meals**	**Mean Select Moderate Fish Meals[1]**	**Mean Canned Tuna Fish Meals**	**Mean Fish Meals**	**Mean Fish Meals, Excluding Low Hg Meals[2]**	**Mean Moderate Hg Fish Meals[3]**
All Participants	499	22.7	5.1	4.2	19	15.7	6
Age							
16-30	200	22.3	4.9	3.7	19.3	15.5	5.7
31-40	158	21.5	4.5	3.9	18.4	14.2	5.4
41-50	141	24.8	6.2	5.4	21.9	17.7	6.9
f-value		0.49	0.17	0.08	0.35	0.24	0.33
Education							
<high school	44	24.5	4.5	5.5	20.9	17.2	5.4
High school/ some college	197	22.3	5.6	4.6	19.2	15.5	6.6
College degree	181	24.6	5.5	4	21.9	17.3	6.1
Post graduate work	77	18.5	3.4	2.8	15.6	11.5	4.3
f-value		0.3	0.19	0.14	0.17	0.12	0.28
N Palm Size Fish Pieces/Meal							
Don't eat fish	25	2.1	0	1.7	1.9	1.8	0
<1	36	12.2	1	4.2	11.1	7.2	1.4
1	139	21.5	4.2	4	18.1	13.7	5.1
2	194	26.6	6	4.5	22.9	18.5	7
3	71	25.2	6.7	4.3	22.7	18.1	7.5
4	31	27.5	9.3	5.1	25.2	22.6	9.8
f-value		<0.0001	<0.0001	0.54	<0.0001	<0.0001	<0.0001
Season of Enrollment[4]							
Winter	98	17.4	2.6	3.6	14.2	10.1	3.2
Spring	97	20.6	3.8	4.6	17.5	13.5	4.8
Summer	136	27.2	6	4.1	23.9	18.7	7.2
Fall	168	23.5	6.7	4.4	20.9	17.8	7.2
f-value		0.02	0.0002	0.78	0.003	0.0009	0.001

[1]Select moderate Hg fish = walleye, northern pike, bass, lake trout
[2]Low Hg fish = shellfish, tilapia, salmon and fish sticks
[3]Moderate Hg fish = walleye, northern pike, bass, lake trout, halibut, and tuna steak/fillet
[4]Winter = December–February, Spring = March–May, Summer = June-August, Fall = September-November
P-values for percentages from Fisher's exact tests and for continuous variables from ANOVA

Biomonitoring

Participant characteristics evaluated for predicting blood mercury and plasma EPA and DHA included: age, education level, season of clinic visit, and usual number of palm-sized pieces of fish per meal. Omega-3 supplement and supplemented food consumption frequency were also included for EPA and DHA.

Bivariate associations of participant characteristics with blood mercury, blood mercury greater than 5.8 µg/L (which is the blood level equivalent to the US EPA's Reference Dose (RfD) for MeHg of 0.1 µg/kg/day), and fatty acids are shown in Table 6. Mean blood mercury increased with age, education level, and larger meal size. It was lowest in blood collected in the spring and highest in fall samples. Three percent of all participants had elevated blood mercury; this percentage was higher in older women and those who ate larger meals. Methylmercury in blood samples with total mercury > 5.8 µg/L ranged from 86 to 100 percent of total mercury, with an average of 95 percent. These results confirm fish as the source of exposure, as mercury from sources such as thermometers or skin lightening creams would be in inorganic or elemental forms. Fetal blood mercury has been reported (Stern and Smith 2003) to average 1.6 times higher than maternal blood mercury. Blood mercury in ten percent of FISH participants was above the level equivalent to the RfD divided by 1.6.

Associations of participant characteristics with fatty acids were not consistent for DHA and EPA. Both DHA and EPA increased with education level and omega-3 supplement use, while only EPA was positively associated with age and only DHA increased with larger meal size. Levels of DHA and EPA did not differ by season of sampling.

Table 6. Associations of Participant Characteristics with Blood Mercury and Plasma Fatty Acids

Characteristic	N	Mean Blood Hg, µg/L	Proportion with Blood Hg >5.8 µg/L	Mean Plasma DHA, µg/mL	Mean Plasma EPA, µg/mL	Mean Plasma EPA & DHA, µg/mL
***All Participants*[1]**	499	1.67	3.00%	47	16.5	63.4
Age						
16-30	200	1.35	0%	45.2	13.3	58.5
31-40	158	1.55	3.20%	48.4	16.9	65.3
41-50	141	2.27	7.10%	47.9	20.5	68.4
f-value		<0.0001	0.0002	0.35	<0.0001	0.01
Education						
<high school	44	1.01	0%	36.4	11.7	48.1
High school/ some college	197	1.54	4.10%	43.3	15.1	58.4
College degree	181	1.98	2.20%	49.7	17.6	67.3
Post graduate work	77	1.65	3.90%	55.9	19.9	75.8
f-value		0.02	0.51	<0.0001	0.0005	<0.0001
N Palm Size Fish Pieces/Meal						
Don't eat fish	25	0.44	0%	29.3	10.9	40.2
<1	36	1.04	0%	48	17.4	65.3
1	139	1.38	0.70%	48.7	16.3	65
2	194	1.88	3.60%	47.2	17.3	64.5
3	71	1.91	5.40%	46.4	16.2	62.6
4	31	2.84	9.70%	46.2	15.8	62
f-value		<0.0001	0.06	0.004	0.24	0.01
Omega-3 Supplement or Supplemented Food						
Never	223	1.78	3.90%	42.6	14.4	57
Occasionally	61	1.41	0%	42.9	13.7	56.5
Weekly	123	1.56	1.60%	43.9	15.1	59
Daily	82	1.75	4.90%	66.5	26.3	92.8
f-value		0.52	0.25	<0.0001	<0.0001	<0.0001
***Season of Enrollment*[2]**						
Winter	98	1.63	2.00%	49	16.3	65.3
Spring	97	1.22	2.10%	42.9	15.4	58.4
Summer	136	1.57	2.90%	49.1	17.5	66.5
Fall	168	2.04	4.20%	46.4	16.2	62.6
f-value		0.009	0.81	0.15	0.59	0.22

[1]N=490 for DHA and EPA analyses

[2]Winter = December–February, Spring = March–May, Summer = June-August, Fall = September-November

P-values for percentages from Fisher's exact tests and for continuous variables from ANOVA

Associations of Plasma Fatty Acids with Fish Consumption and Participant Characteristics

Associations of fish consumption metrics and participant characteristics with geometric mean plasma fatty acids were examined using linear regression models fitted with the natural log-transformed fatty concentration as the outcome (Tables 7 and 8).

For participant characteristics, use of omega-3 supplements or supplemented foods explained the largest proportion of variability (R-square) for DHA and DHA & EPA, while age explained the largest proportion of variability in EPA (Table 7). For fish metrics, R-square values were larger for DHA than EPA and, in general, fish meals in the past week explained a larger proportion of variability in DHA than fish meals in the past 3 months. Interestingly, the proportions of variability in DHA, EPA, and DHA & EPA explained by a variable indicating fish consumer versus non-consumer were generally comparable to the proportions explained by detailed information about fish and shellfish meals in the past week.

Individual fish species and fish metrics that best predicted blood mercury were identified using stepwise selection and included adjustment for age, education, season, portions/meal, and omega-3 supplement use (Table 8). For fish metrics, stepwise selection identified both fish and shellfish meals in the past week and fish consumer versus non-consumer for DHA and DHA & EPA, explaining 24% and 25% variability in fatty acid levels, respectively, with adjustment for covariates. We were able to explain only 18% of variability in EPA using fish metrics and covariates. For individual fish species, stepwise selection identified different fish for each plasma fatty acid. The largest proportion of variation (25%) was explained for DHA & EPA by lake herring, salmon, lake trout, tilapia (negative predictor), fish consumer versus non-consumers, and covariates.

Table 7. Bivariate Associations of Fish Consumption[1] with Geometric Mean Plasma Fatty Acids (n=490)

Characteristic	% Change in Geometric Mean DHA (95% CI)	Model R-square	% Change in Geometric Mean EPA (95% CI)	Model R-square	% Change in Geometric Mean DHA+EPA (95% CI)	Model R-square
Age, years	0.6 (0.2, 1.0)	0.02	2.4 (1.8, 3.1)	0.10	1 (0.6, 1.4)	0.04
Education category	9.0 (6.0, 12.1)	0.07	12.8 (7.7, 18.2)	0.05	9.7 (6.6, 12.8)	0.08
Number palm size fish portion/meal	5.2 (1.2, 9.4)	0.01	4.4 (-2.1, 11.3)	0.04	4.9 (0.8, 9.1)	0.01
Omega-3 supplement category	11.1 (7.6, 14.7)	0.08	15.5 (9.5, 21.9)	0.05	12.2 (8.6, 15.9)	0.09
Summer versus Spring	15.6 (2.8, 30.0)	0.02	9.6 (-9.6, 33.0)	0.003	14.9 (1.9, 29.5)	0.01
Fall versus Spring	9.3 (-2.3, 22.3)		6.7 (-11.4, 28.3)		9.0 (-2.7, 22.3)	
Winter versus Spring	17.8 (4.0, 33.6)		12.1 (-8.9, 37.8)		16.6 (2.6, 32.5)	
Fish consumer versus non-consumer	65.3 (38.7, 97.1)	0.06	58.7 (18.2, 113.0)	0.02	60.3 (33.9, 92.0)	0.05
EMR Screening Questions						
Fish and shellfish meals	7.8 (4.2, 11.6)	0.04	11.2 (5.1, 17.6)	0.03	8.4 (4.7, 12.2)	0.04
Select moderate Hg fish meals[2]	8.6 (0.8, 16.9)	0.01	11.1 (-1.6, 25.5)	0.01	9.0 (1.1, 17.6)	0.01
Canned tuna fish meals (n=148)	12.2 (-3.0, 29.9)	0.02	-7.9 (-27.5, 17.1)	0.003	11.2 (-4.1, 29.0)	0.01
Detailed fish consumption in past 3 months						
Fish and shellfish meals	4.5 (2.4, 6.6)	0.04	4.2 (0.8, 7.8)	0.01	4.4 (2.3, 6.6)	0.03
Select moderate Hg fish meals[2]	7.3 (1.0, 1.4)	0.01	8.8 (-1.6, 20.3)	0.01	7.8 (1.2, 14.7)	0.01
Canned tuna fish meals	4.7 (-2.7, 12.7)	0.003	1.7 (-9.9, 14.7)	0.0001	4.2 (-3.3, 12.3)	0.002
Fish meals, excluding shellfish	4.9 (2.5, 7.4)	0.03	4.9 (0.9, 9.0)	0.01	4.9 (2.4, 7.5)	0.03
Fish meals, excluding low Hg meals[3]	5.6 (2.8, 8.5)	0.03	5.6 (0.9, 10.4)	0.01	5.7 (2.8, 8.6)	0.03
All moderate Hg fish meals[4]	7.4 (1.7, 13.5)	0.01	7.6 (-1.7, 17.8)	0.005	7.4 (1.6, 13.6)	0.01
Detailed fish consumption in past week						
Fish and shellfish meals	5.9 (3.7, 8.0)	0.06	6.4 (2.9, 10.0)	0.03	6.1 (3.0, 8.3)	0.06
Select moderate Hg fish meals[2]	9.5 (3.5, 15.9)	0.02	9.0 (-0.7, 19.6)	0.01	10.0 (3.9, 16.5)	0.02
Canned tuna fish meals	3.7 (-2.5, 10.4)	0.003	-3.4 (-12.8, 7.0)	0.001	2.8 (-3.5, 9.6)	0.002
Fish meals, excluding shellfish	7.0 (4.5, 9.6)	0.06	7.3 (3.1, 11.6)	0.02	7.3 (4.7, 9.9)	0.06
Fish meals, excluding low Hg meals[3]	7.6 (4.5, 10.8)	0.05	6.2 (1.2, 11.6)	0.01	7.5 (4.4, 10.8)	0.04
All moderate Hg fish meals[4]	10.4 (4.8, 16.3)	0.03	9.0 (0, 18.9)	0.001	10.6 (4.8, 16.6)	0.03

[1]All fish consumption variables scaled to meals/week to allow comparison across metrics. Estimates represent the proportional change in the geometric mean of plasma fatty acids for each 1 meal increase in fish/shellfish consumption or a change in 1 category of the covariate. [2]Select moderate Hg fish = walleye, northern pike, bass, and lake trout. [3]Low Hg fish = shellfish, tilapia, salmon, and fish sticks. [4]All moderate Hg fish = walleye, northern pike, bass, lake trout, halibut and tuna steak/fillet.

Table 8. Multivariable Associations of Fish Consumption[1] with Geometric Mean Plasma Fatty Acids (n=490)

Characteristic	% Change in Geometric Mean DHA (95% CI)	Model R-square	% Change in Geometric Mean EPA (95% CI)	Model R-square	% Change in Geometric Mean DHA+EPA (95% CI)	Model R-square
Multivariable Associations with Summary Fish Variables [2,3]						
Detailed fish consumption in past week						
Fish and shellfish meals	5.4 (3.5, 7.4)	0.24	8.1 (4.4, 12.0)	0.19	5.6 (3.6, 7.7)	0.25
Canned tuna fish meals			-13.6 (-22.0, -4.2)		-	
Other fish consumption variables						
Fish consumer versus non-consumer	38.4 (15.7, 65.7)		-		32.0 (10.1, 58.4)	
Multivariable Associations with Individual Fish Species[2,4]						
Lake Herring	12.4 (1.8, 24.0)	0.22	19.2 (1.0, 40.8)	0.18	10.8 (0.1, 22.5)	0.25
Salmon	-				10.1 (0.6, 20.4)	
Lake Trout	14.6 (2.3, 28.4)		21.7 (0.5, 47.4)		16.2 (3.6, 30.4)	
Tilapia	-				-22.5 (-39.3, -1.2)	
Other fish consumption variables						
Fish consumer versus non-consumer	42.2 (18.6, 70.5)		-		34.0 (11.6, 61.0)	

[1]All fish consumption variables scaled to meals/week to allow comparison across metrics. Estimates represent the proportional change in the geometric mean of plasma fatty acids for each 1 meal increase in fish/shellfish consumption or a change in 1 category of the covariate.
[2]Adjusted for age, education, season of test, omega-3 supplement and fortified food use, and number of fish pieces/meal.
[3]Identified using stepwise selection from fish metrics for past week and past 3 months.
[4]Identified using stepwise selection from individual fish species consumed during past 3 months.

Evaluation of Reported Fish Consumption for Predicting Mercury Exposure

Associations of fish metrics and participant characteristics with geometric mean blood mercury were analyzed using linear regression models fitted with the natural log-transformed blood Hg concentration as the outcome (Table 9). The proportional increase in geometric mean blood Hg per fish meal was largest for the select moderate fish metric EMR Screening Question, DQ-calculated select moderate Hg fish meals in the past 3 months, and DQ-calculated all moderate fish meals in the past 3 months (80%, 71%, and 67% increase per fish meal, respectively).

Individual fish species and fish metrics that best predicted blood mercury were identified using stepwise selection and included adjustment for age, education, season, and fish pieces/meal (Table 10). For fish metrics, stepwise selection identified both all moderate Hg fish meals in the past 3 months and fish meals (excluding low Hg meals) in the past week as significant predictors of blood Hg. With adjustment for covariates, the model explained 34% of the variance in natural log-transformed blood Hg. For individual fish species, stepwise selection identified walleye, lake trout, canned tuna, tuna steaks, and fish sticks (negative predictor) as significant predictors of blood Hg. With adjustment for covariates, the model explained 35% of the variance in natural log-transformed blood Hg.

Table 9. Bivariate Associations of Fish and Shellfish Consumption[1] with Geometric Mean Blood Hg (n=499)

Characteristic	% Change in Geo. Mean Hg (95% CI)	Model R-square
Age, years	2.3 (1.4, 3.1)	0.05
Education category	9.3 (3.0, 16.0)	0.02
Number palm size fish pieces/meal	25.7 (16.1, 36.0)	0.06
Summer versus Spring	2.8 (-18.8, 30.1)	0.04
Fall versus Spring	49.1 (18.9, 87.0)	
Winter versus Spring	44.4 (12.0, 86.2)	
EMR Screening Questions		
Fish and shellfish meals	29.0 (20.5, 38.3)	0.10
Select moderate Hg fish meals[2]	80.3 (55.9, 108.6)	0.11
Canned tuna fish meals (n=148)	60.9 (21.4, 113.3)	0.07
Detailed fish consumption in past 3 months		
Fish and shellfish meals	18.9 (14.2, 23.8)	0.13
Select moderate Hg fish meals[2]	71.4 (52.2, 93.0)	0.14
Canned tuna fish meals	26.9 (9.6, 46.9)	0.02
Fish meals, excluding shellfish	22.4 (16.9, 28.2)	0.13
Fish meals, excluding low Hg meals[3]	29.2 (22.5, 36.2)	0.15
All moderate Hg fish meals[4]	67.3 (50.5, 86.0)	0.16
Detailed fish consumption in past week		
Fish and shellfish meals	19.4 (14.7, 24.3)	0.13
Select moderate Hg fish meals[2]	57.9 (41.3, 76.6)	0.12
Canned tuna fish meals	26.8 (11.6, 44.1)	0.03
Fish meals, excluding shellfish	24.5 (18.8, 30.4)	0.15
Fish meals, excluding low Hg meals[3]	32.4 (25.0, 40.2)	0.16
All moderate Hg fish meals[4]	54.2 (39.1, 70.9)	0.12

[1]Fish consumption variables scaled to meals/week to allow comparison across metrics. Estimates are presented as exponentiated model coefficients, which can be interpreted as the proportional change in the geometric mean Hg associated with an increase in 1 fish meal/week or a change in 1 category of the covariate.
[2]Select moderate Hg fish = walleye, northern pike, bass, and lake trout.
[3]Low Hg fish = shellfish, tilapia, salmon, and fish sticks.
[4]All moderate Hg fish = walleye, northern pike, bass, lake trout, halibut, and tuna steak/fillet.

Table 10. Multivariable Associations of Fish and Shellfish Consumption[1] with Geometric Mean Blood Hg (n=499)

Characteristic	% Change in Geo. Mean Hg (95% CI)	Model R-square
Multivariable Associations with Summary Fish Variables[2,]		
Detailed fish consumption in past 3 months		
All moderate Hg fish meals[4]	36.2 (21.1, 53.2)	0.34
Detailed fish consumption in past week		
Fish meals, excluding select low Hg meals[5]	19.1 (11.7, 26.9)	
Multivariable Associations with Individual Fish Species[3]		
Walleye	30.2 (3.0, 64.7)	0.35
Lake Trout	110.7 (69.9, 161.3)	
Tuna, canned	24.2 (8.9, 41.6)	
Tuna, not canned	82.6 (29.9, 156.9)	
Fish sticks	-51.8 (-65.9, -31.9)	

[1]Fish consumption variables scaled to meals/week to allow comparison across metrics. Estimates are presented as exponentiated model coefficients, which can be interpreted as the proportional change in the geometric mean Hg associated with an increase in 1 fish meal/week or a change in 1 category of the covariate.
[2]Identified using stepwise selection from fish metrics for past week and past 3 months, and adjusted for age, education, season of test, and number of fish pieces/meal.
[3] Identified using stepwise selection from individual fish species consumed during past 3 months, and adjusted for age, education, season of test, and number of fish pieces/meal.
[4]All moderate Hg fish = walleye, northern pike, bass, lake trout, halibut, and tuna steak/fillet.
[5]Low Hg fish = shellfish, tilapia, salmon, and fish sticks.

Associations of fish consumption metrics and covariates with blood mercury greater than 5.8 µg/L were evaluated using logistic regression. Odds of elevated blood Hg per increase in one fish meal was significantly increased for all fish metrics, except for canned tuna fish meals (Table 11). DQ-calculated select moderate Hg fish meals in the past week, in the past 3 months, and from the EMR screening question had the strongest associations with elevated blood Hg (OR per meal=3.56, 3.06, and 2.98, respectively), followed by all moderate Hg fish (walleye, northern pike, bass, lake trout, halibut, and tuna steaks) meals in the past week and in the past month (OR per meal=2.95 and 2.65, respectively). Fish and shellfish meals, fish meals excluding shellfish, and fish meals excluding low Hg fish (shellfish, tilapia, salmon, and fish sticks) were not as strongly associated with elevated blood Hg compared to the metrics for moderate Hg fish species.

Individual fish species and fish metrics that best predicted elevated blood mercury were identified using stepwise selection and included adjustment for age, education, season, and portions/meal (Table 11). For fish metrics, odds of elevated blood Hg was associated with select moderate Hg fish meals in the past week (OR=3.80, 95% CI=2.03, 7.12). For individual species, tuna steaks and lake trout meals were associated with elevated blood Hg.

Table 11. Odds Ratios for Blood Hg >5.8 µg/L by Participant Characteristics and Fish Consumption[1] (n=499)

Characteristic	OR Blood Hg >5.8 µg/L (95% CI)	P-value
Bivariate Associations		
Age, years	1.16 (1.07, 1.25)	0.0003
Education category	1.15 (0.79, 1.68)	0.48
Number palm size fish pieces/meal	2.21 (1.33, 3.66)	0.002
Summer versus Spring	1.44 (0.26, 8.02)	0.68
Fall versus Spring	2.07 (0.42, 10.15)	0.37
Winter versus Spring	0.99 (0.14, 7.17)	0.99
EMR Screening Questions		
Fish and shellfish meals	1.38 (1.06, 1.81)	0.02
Select moderate Hg fish meals[2]	2.98 (1.78, 4.98)	<0.0001
Canned tuna fish meals (n=148)	1.30 (0.22, 7.93)	0.77
Detailed fish consumption in past 3 months		
Fish and shellfish meals	1.26 (1.08, 1.47)	0.004
Select moderate Hg fish meals[2]	3.06 (1.80, 5.21)	<0.0001
Canned tuna fish meals	1.28 (0.61, 2.69)	0.51
Fish meals, excluding shellfish	1.37 (1.15, 1.64)	0.0004
Fish meals, excluding low Hg meals[3]	1.45 (1.19, 1.77)	0.0002
All moderate Hg fish meals[4]	2.65 (1.74, 4.05)	<0.0001
Detailed fish consumption in past week		
Fish and shellfish meals	1.26 (1.08, 1.48)	0.004
Select moderate Hg fish meals[2]	3.56 (2.11, 6.02)	<0.0001
Canned tuna fish meals	1.28 (0.64, 2.54)	0.49
Fish meals, excluding shellfish	1.43 (1.18, 1.74)	0.0003
Fish meals, excluding low Hg meals[3]	1.67 (1.30, 2.14)	<0.0001
All moderate Hg fish meals[4]	2.95 (1.88, 4.64)	<0.0001
Multivariable Associations with Fish Metrics Variables[5,6]		
Detailed fish consumption in past week		
Select moderate Hg fish meals/w[2]	3.80 (2.03, 7.12)	<0.0001
Multivariable Associations with Individual Fish Species[5,7]		
Lake Trout	8.91 (2.96, 26.82)	<0.0001
Tuna, not canned	9.04 (1.90, 42.88)	0.006

[1]All fish consumption variables scaled to meals/week for comparison. [2]Select moderate Hg fish = walleye, northern pike, bass, and lake trout. [3]Low Hg fish = shellfish, tilapia, salmon, and fish sticks. [4]All moderate Hg fish = walleye, northern pike, bass, lake trout, halibut, and tuna steak/fillet. [5]Adjusted for age, education, season of test, and number of fish pieces/meal. [6]Identified using stepwise selection from fish metrics for past week and past 3 months. [7]Identified using stepwise selection from individual fish species consumed during past 3 months.

The utility of fish metrics as a screening test predicting Hg were explored considering a value of Hg ≥ 5.8 µg/L as the "gold standard." Sensitivity and specificity were examined for individual fish metrics and for combinations of fish metrics considered simultaneously. Receiver operator characteristic (ROC) curves were created by plotting the calculated sensitivity against 1-specificity.

Sensitivity and specificity were similar for the "Fish and shellfish meals per week" and "Select moderate Hg fish meals per month" EMR Screening Questions. Cut points of one meal per week and one meal per month for the two questions, respectively, were selected based on sensitivity. Sensitivity declines as the cut off increases.

DQ-calculated screening questions had higher area under the ROC curve and sensitivity than corresponding EMR Screening Questions (see Table 12, Figures 1 and 2). Select moderate and all moderate Hg fish meals per month questions had similar area under ROC curve, sensitivity, and specificity and the highest overall area under ROC curve and sensitivity. Reported fish consumption could be higher for the DQ-calculated question on Select moderate Hg fish meals because it included all lake trout; whereas, the EMR screening question on Select moderate Hg fish meals included only lake trout from Lake Superior.

Table 12. Sensitivity and specificity of fish consumption questions for identifying women with Blood Hg >5.8 µg/L

Screening Test	Area under ROC curve[1]	Sensitivity	Specificity	PPV	NPV
EMR Screening Questions					
≥1 Fish and shellfish meals/w	0.74	0.87	0.49	0.05	0.99
≥2 Fish and shellfish meals/w		0.60	0.79	0.08	0.98
≥1 Select moderate Hg fish meals/m[2]	0.76	0.80	0.45	0.04	0.99
≥2 Select moderate Hg fish meals/m[2]		0.67	0.69	0.06	0.99
Detailed fish consumption in past 3 months					
≥1 Fish and shellfish meals/w	0.76	0.87	0.42	0.04	0.99
≥1 Fish meals, excluding shellfish/w	0.78	0.87	0.47	0.05	0.99
≥1 Canned tuna fish meals/w	0.53	0.13	0.92	0.05	0.97
≥1 Fish meals, excluding select low Hg meals/w[3]	0.82	0.87	0.59	0.06	0.99
≥1 Select moderate Hg fish meals/m[2]	0.90	1.00	0.57	0.07	1.00
≥1 All moderate Hg fish meals/m[4]	0.89	1.00	0.51	0.06	1.00
Detailed fish consumption in past week					
≥1 Fish and shellfish meals/w	0.73	0.93	0.38	0.04	0.99
≥1 Fish meals, excluding shellfish/w	0.76	0.93	0.42	0.05	0.99
≥1 Canned tuna fish meals/w	0.53	0.27	0.79	0.04	0.97
≥1 Fish meals, excluding select low Hg meals/w[3]	0.76	0.87	0.48	0.05	0.99
≥1 Select moderate Hg fish meals/w[2]	0.79	0.73	0.79	0.10	0.99
≥1 All moderate Hg fish meals/w[4]	0.77	0.73	0.76	0.09	0.99

[1]A larger area under the ROC curve indicates a greater test accuracy.

[2]Select Hg fish = walleye, northern pike, bass, or Lake Superior lake trout.

[3]Low Hg fish = shellfish, tilapia, salmon, and fish sticks.

[4]All moderate Hg fish = walleye, northern pike, bass, lake trout, halibut, and tuna steaks/fillets.

Figure 1. ROC Curve for moderate Hg fish meals from the detailed questionnaire (walleye, northern pike, bass, or Lake Superior lake trout) for identifying women with Blood Hg >5.8 µg/L. At a cut point of ≥ one meal/month, the sensitivity=1.00, specificity=0.36, PPV=0.05, and NPV=1.00.

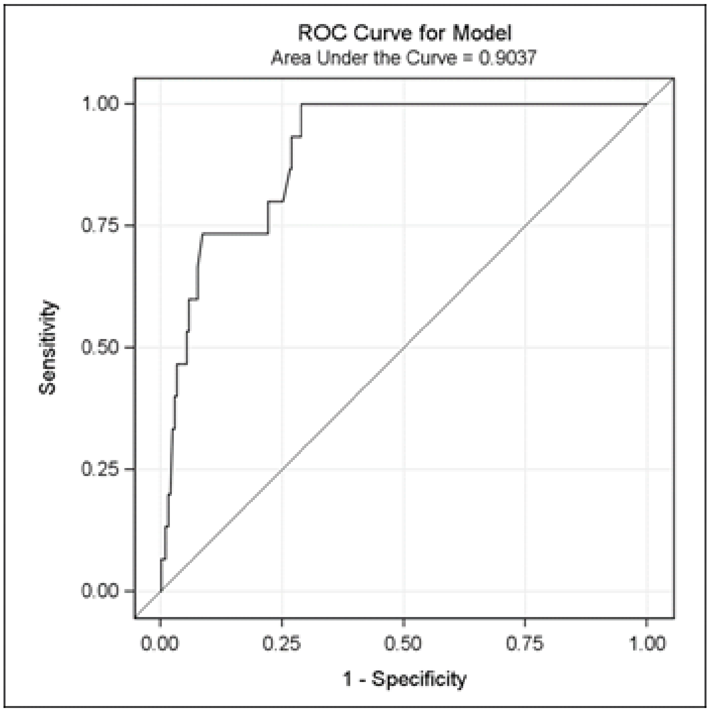
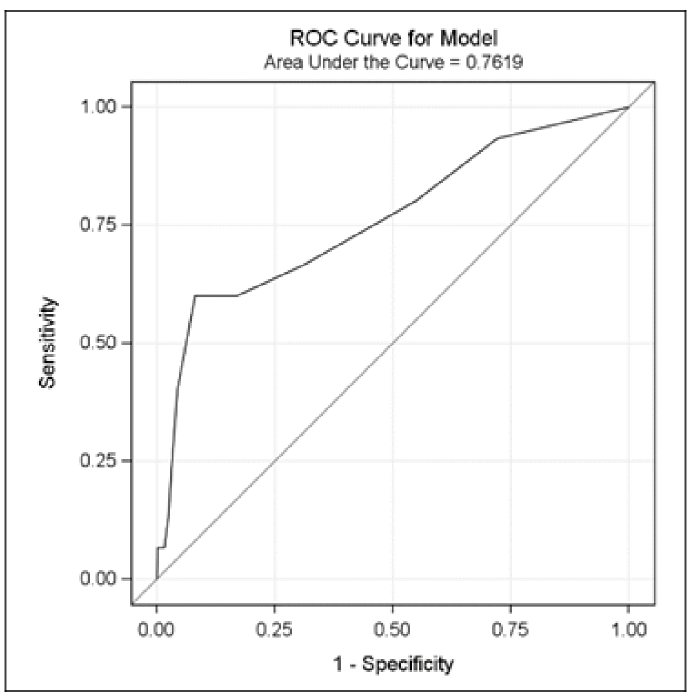


Figure 2. ROC Curve for moderate Hg fish meals from the EMR question (walleye, northern pike, bass, or Lake Superior lake trout) for identifying women with Blood Hg >5.8 µg/L. At a cut point of ≥ one meal/month, the sensitivity=0.80, specificity=0.45, PPV=0.04, and NPV=0.99.



Asking questions in parallel increased the sensitivity compared with asking the EMR Screening Questions individually. DQ-calculated questions on Select moderate or All moderate Hg fish meals are better individually than in parallel with another questions; specificity goes down when asked in parallel (Table 13). Any combination with either DQ-calculated Select moderate or All moderate Hg fish meals has sensitivity of 1 (Table 14).

Table 13. Parallel testing[1] of EMR questions: Sensitivity and specificity of fish consumption questions for identifying women with Blood Hg >5.8 µg/L

EMR Screening Test 1	EMR Screening Test 2	Sensitivity	Specificity	PPV	NPV
≥1 fish or shellfish meal/w	≥1 Select moderate Hg fish meal/m	0.93	0.31	0.04	0.99
≥1 fish or shellfish meal/w	≥2 Select moderate Hg fish meal/m	0.87	0.44	0.05	0.99
≥2 fish or shellfish meal/w	≥1 Select moderate Hg fish meal/m	0.80	0.41	0.04	0.99
≥2 fish or shellfish meal/w	≥2 Select moderate Hg fish meal/m	0.73	0.60	0.05	0.99

[1]For parallel testing, net sensitivity was calculated using those identified as positive by either test, and net specificity was calculated using those identified as negative by both tests.

Table 14. Parallel testing[1] of detailed questionnaire: Sensitivity and specificity of fish consumption questions for identifying women with Blood Hg >5.8 µg/L

Screening Test 1	Screening Test 2	Sensitivity*	Specificity	PPV	NPV
≥1 fish or shellfish meal/w	≥1 Select moderate Hg fish meal/m	1.00	0.36	0.05	1.00
≥1 fish or shellfish meal/w	≥1 All moderate Hg fish meal/m	1.00	0.35	0.05	1.00
≥1 fish meal/w	≥1 Select moderate Hg fish meal/m	1.00	0.39	0.05	1.00
≥1 fish meal/w	≥1 All moderate Hg fish meal/m	1.00	0.37	0.05	1.00
≥1 fish, excluding low Hg fish meal/w	≥1 Select moderate Hg fish meal/m	1.00	0.45	0.05	1.00
≥1 fish, excluding low Hg fish meal/w	≥1 All moderate Hg fish meal/m	1.00	0.42	0.05	1.00
≥1 canned tuna fish meal/w	≥1 Select moderate Hg fish meal/m	1.00	0.53	0.06	1.00
≥1 canned tuna fish meal/w	≥1 All moderate Hg fish meal/m	1.00	0.47	0.06	1.00

[1]For parallel testing, net sensitivity was calculated using those identified as positive by either test, and net specificity was calculated using those identified as negative by both tests.
*Any combination with either select moderate or all moderate mercury fish has sensitivity =1

Biomonitoring versus Hg Exposure Estimated from Fish Consumption

Fish consumption advice for women who are or may become pregnant is based on keeping exposure below the EPA RfD for methylmercury, 0.1 µg meHg/kg bodyweight/day. Mercury intakes estimated from: 1) fish consumption and meal size scaled according to the number of palm-sized pieces of fish per meal reported in the DQ; 2) a body weight of 70.7 kg, which is the average of the median body weights reported for females 20-29, 30-39, and 40-49 (median is 67.7 kg, 72.5 kg, 71.8 kg, respectively) in NHANES 2007-2010 (NHANES 2012); and 3) mean mercury levels in Minnesota and commercial fish, which are on average 4.5 times higher than intake calculated from measured blood Hg, assuming 0.11 µg meHg/kg bw/d is equivalent to 5.8 µg/L in blood. Figures 3 and 4 show the distributions of the estimated mercury intakes.

Figure 3. Mercury Intake (µg/kg/d) Estimated from DQ Reported Fish Consumption, Mean Fish Mercury, and Meal Size versus Number of Participants

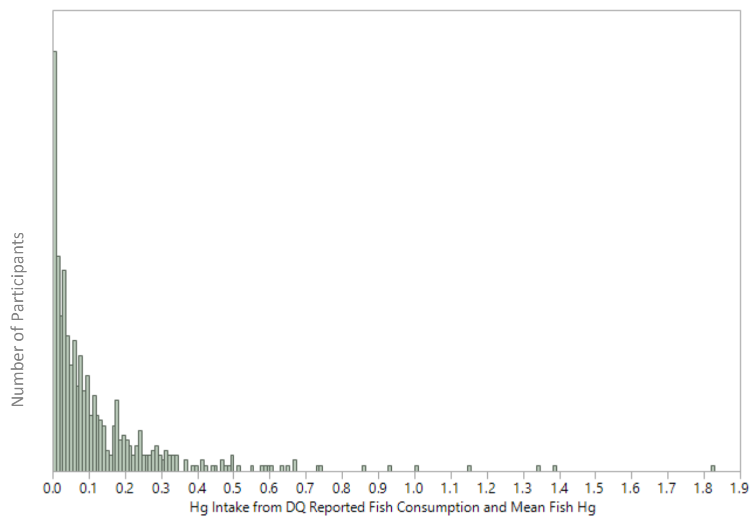
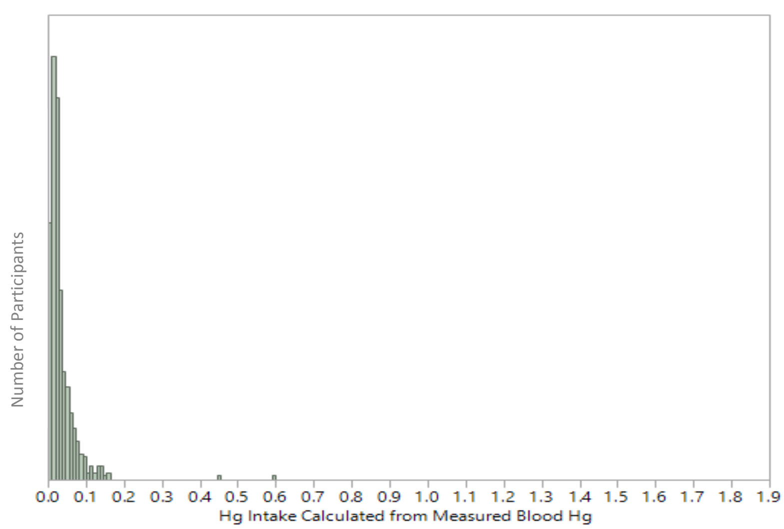


Figure 4. Mercury Intake (µg/kg/d) Calculated from Measured Blood Mercury versus Number of Participants



Variability in fish mercury concentrations explains part of this difference. Table 15 shows the ratios of estimated intake for participants from fish consumption using mean mercury levels, mean minus one standard deviation, mean plus one standard deviation, and mean plus two standard deviations.

Table 15. Ratios of estimated mercury intake from fish consumption and fish mercury

Ratio of estimated exposure for participants	Average Ratio	Maximum Ratio	Minimum Ratio
(Mean fish Hg)/(mean-SD fish Hg)	25	526	1.2
(Mean+SD fish Hg)/(mean fish Hg)	1.8	2.8	1.2
(Mean+2SD fish Hg)/(mean fish Hg)	2.6	4.5	1.4

Comparison of FISH and Lake County Mercury Screening Project (MSP) Results

MSP was a collaborative effort by Lake County Health and Human Services Women, Infants, and Children program (LCHHS WIC) and MDH. The project focused on reducing mercury exposure in women who are or may become pregnant and, therefore, in future babies by raising awareness about risks and benefits of eating fish. MSP is an extension of the FISH. MSP participants answered the same 3 screening questions as FISH participants and provided a blood sample that was tested for mercury. Blood mercury and responses to the EMR screening questions on all fish meals and select moderate fish meals were compared (Table 16). FISH Project participants reporting eating more fish overall and had higher mercury levels on average.

Table 16. Comparison of FISH participants with MSP participants

Comparison	Project	N	Mean	Median	p-value
All fish meals/week	FISH	499	1.1	1	0.008*
All fish meals/week	MSP	121	0.80	0.5	
Select moderate Hg fish meals/month	FISH	499	1.5	1	0.08
Select moderate Hg fish meals/month	MSP	121	0.87	0.5	
Blood Hg (µg/L)	FISH	499	1.7	1.2	<0.0001*
Blood Hg (µg/L)	MSP	121	1.1	0.75	

*significant differences based on t-test from two-sided Wilcoxon Mann Whitney (alpha=0.05)

Comparison with NHANES (National Study Participants)

Fish consumption, blood mercury, and plasma fatty acid results from the FISH Project were compared to results for women age 16-50 from the CDC 2011-2014 National Health and Nutrition Examination Survey (NHANES) (Table 17). Fish meals eaten in the past month were calculated for FISH participants using responses in the DQ. FISH participants had higher plasma EPA and DHA, higher blood mercury, and about the same percent elevated blood mercury as NHANES participants. Overall seafood and finfish consumption were also higher in FISH participants.

Table 17. Comparison of FISH participants with NHANES female participants 16-50 years of age

Variable	Units	Cohort	Years	N	Median	Mean	95% CI	Geo Mean	95% CI	% >5.8 µg/L	95% CI
Blood Hg	µg/L	NHANES	2011-14	2582	0.63	1.19	1.03, 1.35	0.70	0.63, 0.78	2.47	1.54, 3.41
		FISH	2014-15	499	1.16	1.67	1.50, 1.85	1.12	1.03, 1.22	3.01	1.51, 4.50
						p<0.0001		p<0.0001		p=0.47	
Seafood	meals/30d	NHANES	2011-14	3332	2.0	4.4	4.0, 4.9	-	-	-	-
		FISH	2014-15	499	5.5	7.6	6.9, 8.3				
						p=<0.0001					
Fin fish	meals/30d	NHANES	2011-14	3332	1.0	2.6	2.4, 2.9	-	-	-	-
		FISH	2014-15	499	4.7	6.6	6.0, 7.2				
						p<0.0001					
Shellfish	meals/30d	NHANES	2011-14	3332	1.0	1.8	1.6, 2.0	-	-	-	-
		FISH	2014-15	499	0.3	1.0	0.8, 1.2				
						p<0.0001					
Plasma EPA	µg/mL	NHANES	2003-04	490	10.3	12.8	11.6, 14.0	10.9	10.1, 11.7	-	-
		FISH	2014-15	490	13.6	16.5	15.4, 17.5	13.2	12.4, 14.1		
						p<0.0001		p<0.0001			
Plasma DHA	µg/mL	NHANES	2003-04	490	38.1	43.9	40.6, 47.3	39.8	37.1, 42.7	-	-
		FISH	2014-15	490	42.9	47.0	45.0, 48.9	42.6	41.0. 44.3		
						p=0.002		p=0.0006			

NHANES estimates incorporated participant weights and survey design variables.

For FISH study, meals in past month was derived from meals in past 3 months divided by 3.

P-values derived from one-sample t-tests comparing FISH participants to NHANES means for continuous variables and from binomial test for the proportion with blood mercury >5.8 µg/L.

Results at Follow-up: Visit 1 versus Visit 2

Changes in fatty acids levels, mercury exposures, and fish consumption following the initial visit and follow-up visit indicate positive results from participation in the FISH project (Table 18). Comments about the Project from participants at follow-up visits were also encouraging (see Follow-up Evaluation Summary in Appendix I.)

Mercury levels were still below 5.8µg/L in all controls at follow-up clinic visits. Participants with elevated mercury at the initial visit and who self-identified as having potential for future pregnancy were also below 5.8µg/l at follow-up visit. Fatty acid levels were unchanged at follow-up. There was an overall decline in blood mercury at follow-up; the decline was greater for participants with elevated mercury levels at the initial visit. Repeated measures models controlling for season of assessment also showed a significant decline in blood mercury in both groups, with a significantly greater decrease in participants with elevated mercury at the initial visit.

Participants with elevated mercury decreased their consumption of fish species with moderate levels of mercury, and there was an overall decline for all species combined in the one meal per month fish advisory category. No change in low mercury fish was observed. Models adjusting for season of assessment found significant decreases in consumption of fish species with moderate levels of mercury and for all species combined only in participants with elevated mercury at the initial visit (Table 19).

Table 18. Descriptive Statistics for Changes at Follow-up

Fatty Acids	N	Mean difference	StdDev	p-value
Change in DHA, all participants	42	-0.97	15.75	0.5
Change in DHA, controls	27	2.24	13.35	0.6
Change in DHA, elevated Hg	15	-6.74	18.43	0.2
Change in EPA, all participants	42	-2.88	15.36	0.5
Change in EPA, controls	27	-0.83	11.42	0.6
Change in EPA, elevated Hg	15	-6.59	20.62	0.4
Blood Mercury				
Change in Hg, all participants	45	-1.57	2.27	<.0001*
Change in Hg, controls	30	-0.52	0.77	0.0008*
Change in Hg, elevated Hg	15	-3.69	2.80	0.001*
Fish Consumption				
Change in All fish, all participants	45	-11.01	23.07	0.001*
Change in All fish, elevated Hg	15	-17.60	27.74	0.01*
Change in All fish, controls	30	-7.71	20.06	0.08
Change in Walleye, all participants	45	-2.25	6.27	0.02*
Change in Lake Trout, all participants	45	-2.94	5.05	0.0001*
Change in Panfish, all participants	45	0.23	1.48	0.9
Change in Whitefish, all participants	45	-0.16	3.54	0.4
Change in Lake herring, all participants	45	-1.24	9.19	0.3
Change in Canned tuna, all participants	45	-0.93	6.96	0.8
Change in Tuna (steak/fillet), all participants	45	-0.46	2.78	0.7
Change in Northern, all participants	45	-0.86	2.91	0.03*
Change in Bass, all participants	45	0.18	1.56	0.9
Fish Advisory Meal Frequency Categories				
Change in combined meals of species in 2/wk category	45	-0.12	0.56	0.1
Change in combined meals of species in 1/wk category	45	-0.11	0.69	0.2
Change in combined meals of species in 1/mo category	45	-1.95	4.78	0.007*

*significant differences based on Wilcoxon signed-rank test (alpha=0.05)

Table 19. Baseline to 6 month change in mean fish consumption in past 3 months and blood mercury, overall and by blood mercury group (Hg >5.8 µg/L versus ≤ 5.8 µg/L at baseline)

	Both Groups (n=45)[1]		Low Hg group (n=30)[1]		High Hg group (n=15)[1]		Across-Group Change p-value
Variable	**Change**	**p-value**	**Change**	**p-value**	**Change**	**p-value**	
Ln(Blood Mercury)	-0.51	<0.0001	-0.29	0.01	-0.73	<0.0001	0.01
All fish meals	-10.3	0.007	-5.6	0.18	-15.1	0.01	0.16
Low Hg fish meals	-2.3	0.51	-3.1	0.43	-1.5	0.78	0.81
Moderate Hg fish meals	-8.3	<0.0001	-2.8	0.12	-13.9	<0.0001	0.0003

[1]Least square means from repeated measures models adjusting for season of assessment

Discussion and Conclusions

FISH Project Outcomes

FISH Project results are comparable to results from the Mercury in Newborns in the Lake Superior Basin Study. Ten percent of newborns from the north shore area of Minnesota tested in that study had blood levels of mercury that were higher than the level equivalent to the US EPA RfD for methylmercury. Mercury levels were higher in babies born in summer months, suggesting locally caught fish as the source of mercury exposure. In comparison, women who participated in the FISH Project ate more fish in summer and species with moderate levels of mercury, walleye, and lake trout were commonly consumed. Meals of fish that were caught, not purchased, comprised 35 percent of total fish meals. Three percent of women had levels of mercury higher than the level equivalent to the US EPA RfD for methylmercury, and 10% had levels higher than the RfD adjusted for the fetal to maternal blood mercury ratio. Similar to other studies, mercury levels increased with age and higher levels of education (Anderson et al. 2004; Li Et al. 2016). Women who participated in the FISH Project reported eating more fish and had higher blood mercury levels than women in a neighboring north shore community and women in NHANES 2011-2014.

Outcomes from the FISH Project were positive. Reductions in mercury exposures were observed between initial clinic visits and follow-up visits. Levels of beneficial omega-3 fatty acids were unchanged. Women in the project continued to eat fish and consumption of low mercury fish did not decline. Women with elevated mercury levels at the initial visit reported eating fewer meals of moderate mercury fish species at follow-up. SMC and GPHS had 499 female patients participate; collaboration between MDH, SMC/GPHS health care providers, and community members established vital and lasting relationships. The commitment of existing and new patients who chose to participate in this study, together, created a rich and robust learning environment.

SMC and GPHS are committed to educating and ultimately reducing mercury exposure in women who are or may become pregnant. EMR screening questions, modified based on project results, will be included in future prenatal visits at SMC and GPHS. The clinics will reach out to women who might become pregnant through WIC and community outreach and education events.

Results from the FISH Project are being expanded statewide. MDH partnered with HealthPartners, a large Midwestern insurer and medical group, to develop, evaluate, produce, and disseminate through health care systems educational products for women who are or may become pregnant. A mobile-friendly web site and brochures (statewide and community specific) and are being launched May 2017.

Fish Consumption as a Predictor of Blood Mercury

Fish consumption metrics and participant characteristics explained about 35% of variation in measured blood mercury. These results are comparable to a recent study by Li et al (2016). Potential additional sources of variability include: 1) variability in mercury levels in fish; and 2) interindividual variability in absorption, clearance rate, and gut microbiome (Jadán-Piedra et al. 2016).

Guidelines on how much fish is safe to eat are generally based on mean mercury levels in fish. The mean mercury levels of all fish consumed and reported through the detailed questionnaire (DQ) over predicted mercury exposure compared to mercury measured in blood. This result leads to confidence that exposure will be below the RfD if advice is followed.

Screening questions about fish consumption have the potential to result in high sensitivity to identify women with elevated mercury exposure. Therefore, we sought to find combinations of questions that maximized sensitivity while balancing specificity and practical considerations, such as the time required to ask the questions. False positives are expected when using reported fish consumption as a screening tool for predicting high Hg exposure. A perfect prediction in a clinical test is not needed if the harm of the intervention is negligible. The main consequence of false positives is providing information to women who don't have high mercury. The harm of false positives can be minimized through carefully constructed health education that results in continued consumption of fish while choosing to eat species that are low in mercury. Providing education to these women may in fact have the benefit of increased fish consumption by pointing out the reasons to eat fish.

Although biomonitoring remains the most effective test for determining risk among those frequently consuming fish, a brief set of questions that assessed total and moderate high Hg fish meals can be an effective tool in predicting which individuals are likely to be at risk of an elevated blood Hg and allow for targeted education by health care providers. Fish metrics on moderate mercury fish best predict elevated blood mercury. Associations of fish consumption metrics with blood mercury greater than 5.8 µg/L show the odds of predicating elevated mercury with a question on moderate mercury fish was similar using the EMR screening question and the question derived from the DQ; improvement was not seen with additional questions on individual species from the DQ. However, sensitivity and specificity for screening questions derived from DQ data were better than questions in the EMR. Responses to the tested EMR screening questions misclassified 7% of women with elevated Hg as "not at risk." Completing a detailed questionnaire is not practical in a health care setting. Higher sensitivity obtained from the DQ derived screening questions indicate a potential to increase the sensitivity of the EMR screening questions. Focus groups may be useful in refining screening questions.

Two questions in parallel are recommended to screen for high mercury exposures; one question on all fish, excluding shellfish (with a note to include canned tuna); and one question on select moderate Hg fish or all moderate Hg fish, depending on knowledge about community. These questions could also be used to identify women who could benefit from increased consumption of low mercury fish.

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Appendices

Appendix A. FISH Project Protocol

Appendix B. QAPP

Appendix C. Health Care Provider Training Report

Appendix D. Brochures

Appendix E. Promotion Summary

Appendix F. Sample Disposal Documentation

Appendix G. EMR and DQ Response Summaries

Appendix H. Community Events Report

Appendix I. Follow-up Evaluation Summary