STUDENT SUMMER INTERNSHIP TECHNICAL REPORT

Mercury Speciation via Diffusive Gradients in Thin-Films Technology

DOE-FIU SCIENCE & TECHNOLOGY
WORKFORCE DEVELOPMENT PROGRAM

Date submitted:

October 13, 2017

Principal Investigators:

Ripley Raubenolt, DOE Fellow Student
Sarah Solomon, DOE Fellow Student
Florida International University

Dr. Michael Paller, Mentor
Dr. Brian Looney, Mentor
Savannah River National Laboratory

Florida International University Program Director:

Leonel Lagos Ph.D., PMP®

Submitted to:

U.S. Department of Energy
Office of Environmental Management
Under Cooperative Agreement # DE-EM0000598

FIU | Applied Research Center
FLORIDA INTERNATIONAL UNIVERSITY

ABSTRACT

Mercury (Hg) is an environmental pollutant that behaves differently depending on the species present. Methylmercury (MeHg) is one species of mercury that is a harmful bioaccumulative toxin that needs a simple and affordable protocol for monitoring and risk assessment. A diffusive gradient in thin-films (DGT) technology is a rapid economical passive sampling technique capable of monitoring target contaminants in water. This study focused on identifying an optimal DGT for monitoring MeHg. A series of laboratory tests were conducted by selecting DGT probes that: 1) sample total Hg by collecting both inorganic Hg and MeHg into a "standard" spheron-thiol resin layer (LSNB), 2) selectively sample organic MeHg using a hydrophobic collection layer (LSND), and 3) selectively sample MeHg by incorporating reactions to manipulate mercury chemistry along the diffusion path. The standard LNSB DGT was effective in collecting both inorganic Hg and MeHg. The hydrophobic LSND DGT showed a slight preference for MeHg over inorganic Hg; however, it did not exhibit sufficient selectivity for effective-practical monitoring of MeHg. Applying mercury speciation chemistry within the DGT maximized the MeHg uptake while minimizing the uptake of inorganic mercury. The resulting reactive DGT (rDGT) represents an effective and practical technology for selectively sampling MeHg in aqueous systems.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Mercury (Hg), organic and inorganic, is an important persistent-bioaccumulative-toxic environmental pollutant. The mercury species present correlates with characteristics such as toxicity, solubility, mobility and bioavailability (Pelcova et al., 2013). Organic mercury such as methylmercury (MeHg) is one of the most toxic species of mercury effecting human and animal health. Hg in the environment can be found naturally, as well as a result of anthropogenic activities such as mining, Hg manufacture and disposal, and fossil fuel combustion (Fernandez-Gomez, et al., 2011).

Mercury contamination has become a global concern due to its ability to be released into the atmosphere in one location and impact ecosystems thousands of kilometers away. As mercury enters an aqueous system, it is subject to methylation or demethylation (Figure 1). Methylmercury is formed by sulphate reducing methogenic microorganisms. Methylmercury partitions into peripyton, plankton and biota that are eaten by invertebrates and fish. As a result, mercury biomagnifies as it accumulates throughout the food chain.

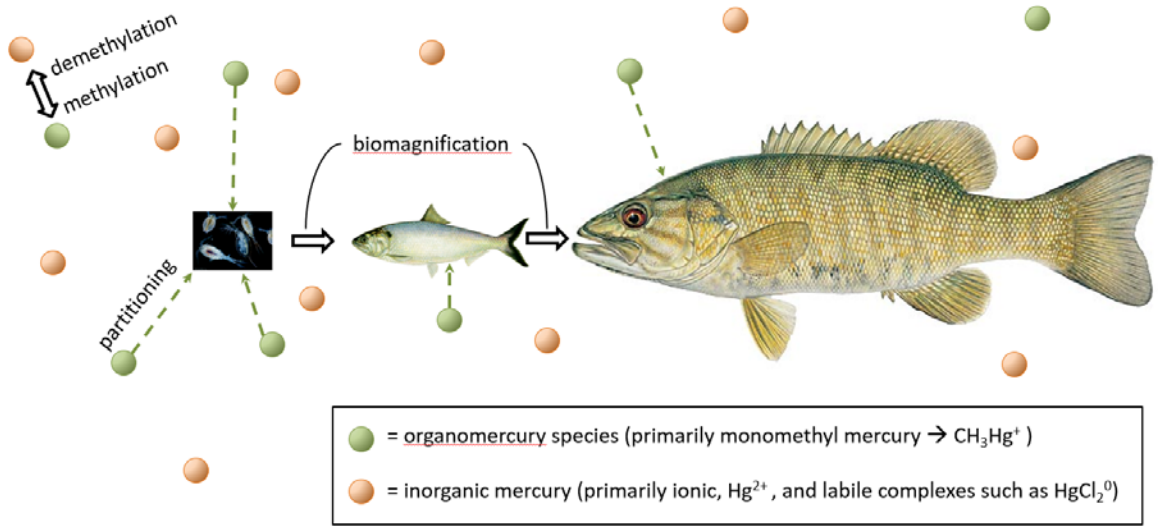

Figure 1. Simplified depiction of the role of mercury speciation in aquatic systems.

Therefore, monitoring both total mercury and mercury species is important to assess due to the impact on human and animal health as well as the environment. Additionally, understanding spatial and seasonal lability of Hg in the environment is important in a technicallt based assessment of risks (Panichev and Panicheva, 2015). To help assess speciation of mercury in aqueous systems, a protocol utilizing diffusive gradients in thin films (DGT) technology, to collect and concentrate total Hg and MeHg contaminants, has the potential reduce costs and increase reliability of monitoring mercury.

A DGT is a passive sampler that accumulates solutes through three encased layers: a filter membrane, a diffusive hydrogel, and a “collection” resin hydrogel. When deployed in a solution, ions diffuse through the first two layers (diffusion zone) and bind to the ion-exchange resin (Fernandez-Gomez et al., 2011). The composition of the two gels correlate with the ions in the

solution being studied (Fernandez-Gomez et al., 2011). For instance, the article *Application of Diffusion Gradient in Thin Films Technique (DGT) for Measurement of Mercury in Aquatic Systems* states that mercury has a high binding capacity with amide groups; thus, polyacrylamide gels cannot be used in the diffusion layer. Instead an agarose diffusion hydrogel replaces it and can be coupled with a spheron-thiol resin that collects total mercury (Docekalova and Divis, 2004). Exposing the DGT probe to a solution for a select amount of time (t) establishes a concentration gradient that represents the amount of metal collected. In utilizing Fick's first law of diffusion, the mass (M) of mercury collected can be related to the solution concentration through the following equation:

$$C_{DGT} = \frac{M\Delta g}{DtA} \quad \text{(Eq. 1)}$$

Where Δg is the thickness of the diffusion layer (cm), D is the diffusion coefficient (cm^2/sec), and A is the surface area exposed to the solution (cm^2) (DGT).

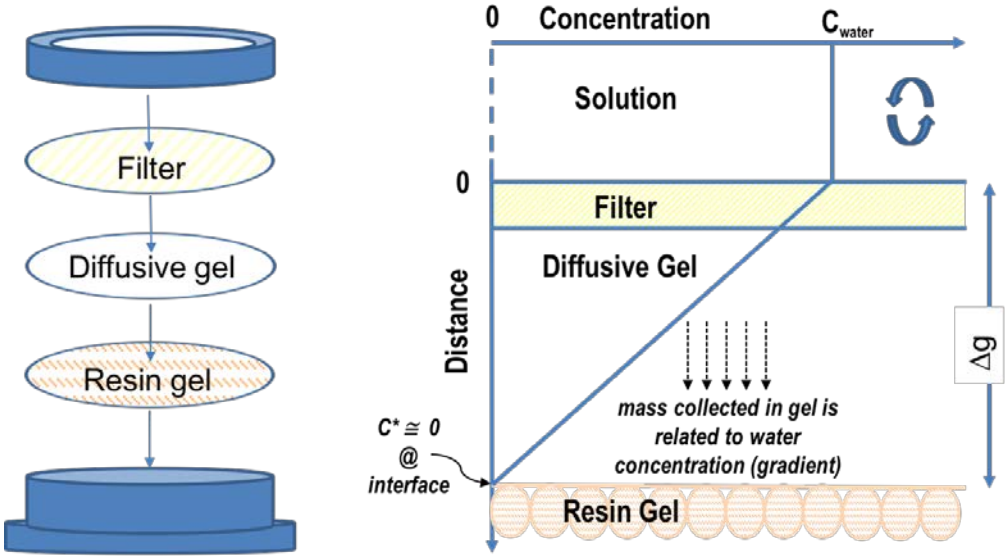

Figure 2. Schematic representation of a DGT and its cross-section illustrating Fick's Law.

The goal of this study is to identify a DGT probe capable of specifically measuring methylmercury as well as develop a protocol to monitor total and organic mercury via in-situ sampling in natural waters using DGT probes. Two approaches were taken. One approach consisted of selecting DGT samplers based on their gel composition. An XAD18 resin and a HLB resin, intended for antibiotics, pesticides, and personal care products, were tested to measure methylmercury based on their ability to collect organic contaminants and methylmercury's octanol-water partition coefficient being similar to that of organic contaminants. A commonly used spheron-thiol resin was selected based on effectiveness in measuring total mercury in previous studies. The second approach was to apply mercury speciation chemistry by modifying the original DGT, creating a reactive DGT containing a spheron-thiol resin by adding an amalgamation layer that was incorporated into the diffusive zone. Here, inorganic mercury was reduced to elemental mercury and then removed through amalgamation. The resin of the DGT samples were analyzed via the Lumex RA915+ Zeeman

effect spectrometer; a pyrolytic technique that converts all mercury to elemental mercury to determine mercury concentration within a sample. Results were then assessed to determine viability.

2. EXECUTIVE SUMMARY

This research work has been supported by the DOE-FIU Science & Technology Workforce Initiative, an innovative program developed by the US Department of Energy's Environmental Management (DOE-EM) and Florida International University's Applied Research Center (FIU-ARC). During the summer of 2017, DOE Fellow interns Sarah Solomon and Ripley Raubenolt spent 10 weeks doing a summer internship at Savannah River Site's Aiken County Technology Laboratory under the supervision and guidance of Dr. Michael Paller and Dr. Brian Looney. The interns' project was initiated on June 5, 2017, and continued through August 11, 2017 with the objective of developing a protocol for utilizing diffusive gradients in thin-films (DGT) for mercury quantification/speciation as well as identifying a DGT probe effective in selectively measuring methylmercury (MeHg).

3. RESEARCH DESCRIPTION

Apparatus

The DGT's were supplied by DGT Research Ltd, Lancaster A2 0QJ, UK. Initially, three different DGT probes were tested: Probe Type 1(LSNBn) containing a spheron-thiol resin intended for total mercury, Probe Type 2 (LSND) containing an XAD18 collection resin intended for antibiotics, and Probe Type 3 (LSNC) containing an HLB collection resin intended for pesticides and personal care products. The resin of the DGT samples were analyzed via the Lumex RA915+ Zeeman effect spectrometer which uses a pyrolytic technique that converts all mercury to elemental mercury to determine mercury concertation within a sample.

Preparation of Standards and Artificial Stream Water for Tests

Standards for MeHg and InHg of 1 mg/L (as Hg) were prepared. Artificial stream water was made in 10 L batches using Nalgene containers by mixing 0.01 g of potassium chloride, 0.307 g of magnesium sulfate heptahydrate, 0.150 g of calcium sulfate dehydrate, and 0.240 g of sodium bicarbonate in NANOpure Diamond deionized water with a conductivity of 18.2 megaohms-cm.

Basic DGT Performance Tests and Sample Campaign Run

To test the validity of the DGT's with the Lumex for measuring mercury concentrations, standard calibration tests were run. DGT resins were spiked with a known amount of mercury standard and placed in the Lumex glass ladles for analysis. This same procedure was also done with standard water samples spiked with the same amount. The standards were tested in the Lumex to create calibration curves for MeHg and InHg. Calibration curves for Probe Type 1 were compared to data obtained in previous experiments. Similar calibration curves were developed for Probes 2 and 3. To become comfortable with future campaign procedures, a test run was set up using four beakers and four DGT probes. A concentration of 1200 ng/L Hg was spiked into each beaker containing 3.8L of gently stirred artificial stream water. DGT's were retrieved at 24, 48, and 113 hours and placed into ladles to be analyzed by the Lumex.

Initiation of Campaign 1 and 2 and Immersion Solution Preparation

Seven 4L beakers were washed with nitric acid and allowed to dry. Artificial stream water solution was poured into each beaker and spiked with Hg standards to create a 1200 mg/L concentration of inorganic mercury, methylmercury, and a solution containing 50% of each; 5.71 mL for methylmercury and 4.56 mL for inorganic mercury. A magnetic stir bar was placed in each beaker which was then placed on a stir plate set on a low speed to allow for gentle mixing. The solutions in each beaker equilibrated before placing the DGT's into the beakers to compensate for mercury stability. For the first campaign run, Probe Types 1 and 2 were tested. For the second campaign run, Probe Type 3 and an older Type 1, for comparison, were tested.

DGT Deployment

The DGT probes were taken out of the cold room (39°F) and removed from their polyethylene bags without contaminating the white face filter membrane. Monofilament was attached to the holes in the base of the DGTs so they could be hung inside the beakers. The probes were then fully immersed into the beakers. Each beaker contained 2 DGTs, for the purpose of duplication and verifying that results were consistent for each probe type. Water samples of each beaker for each campaign were taken (250 mL) and the DGT probes were immersed for 48 hours.

Preparation of Bromine Monochloride for Analysis Procedures

To preserve the samples and convert the organic mercury in the water samples to an elemental state, 1 mL of bromine monochloride (BrCl) was added to each water sample. The reagent was prepared as described in EPA Method 1631. After the water samples were preserved with bromine monochloride, they were analyzed using EPA Method 7470. Water samples representative of the deployment and harvesting were collected during campaigns 1 through 4. The deployment/harvest concentrations were averaged and the resulting values were used in the DGT equation for interpreting the various tests. Water concentrations for the trial run were estimated based on regression of the deployment/harvest measurements of inorganic mercury in the later campaigns (see Appendix A).

Pyrolysis Analysis of Solid Samples using the Lumex 915+

All samples were analyzed using the Lumex RA-915+ Zeeman Effect Spectrometer equipped with a Lumex RP 91C solids (desorption/pyrolysis) attachment. The quartz ladles were lined with aluminum foil and placed in the instrument to bake out any preexisting mercury. Each sample/standard was placed on the aluminum foil and covered with approximately 1.5 g of a mixed powder of sodium carbonate ($NaCO_3$) and copper oxide (CuO) – 50% each by mass. The mixed powder was then placed in the oven at 95°C for 24 hours before use. Use of the powder reduced smoke formation and aided in converting all of the mercury in the samples into elemental mercury for accurate detection. The loaded ladles were placed in the instrument and heated to 700°C for 130sec. Mercury was released into the spectrometer for quantitation.

DGT Processing and Analysis

At the end of the proposed time periods, the DGT's were retrieved from the beakers without touching the white face filter membrane. The probes were then rinsed with deionized water and shaken to remove any surface water still present. The DGT's that were not being analyzed immediately were placed in individual polyethylene bags with minimum air space. Each bag was labeled and stored in the cold room. During analysis, the probes were removed from the cold room and taken out of their individual bags. The cap of the probe was twisted open and the hydrogel resin was obtained. The resin was then placed in a clean glass ladle lined with aluminum foil and covered with the $NaCO_3$ and CuO mix.

After the data was received, the performance of each probe was assessed by estimating an apparent diffusion coefficient, D*, using equation (2). If the DGT is performing well, then D* will be similar to the theoretical value and within the bounds of the values measured in the literature for mercury DGTs. Low values of D* indicate that the DGT is not effectively

collecting the analyte. In this case, a low D* indicates that: 1) the target mercury is not being effectively and completely taken up by the collection gel and/or, 2) mercury is being trapped in the diffusion zone (e.g, by sorption to the filter or gel).

$$D^* = \frac{M\Delta g}{\bar{C}_{dgt} t A} \quad \text{(Eq. 2)}$$

Where Δg is the sum of the diffusive gel layer thickness and the filter membrane thickness, M is the mass of Hg collected onto the gel, D is the diffusion coefficient of Hg in the gel, t is the deployment time, and A is the exposure area.

Preparation of Modified DGT's for More Efficient MeHg Uptake

Stannous Chloride

The data received from campaigns 1 and 2 suggested that the spheron-thiol resin could not be used to selectively uptake MeHg as the strength of the differential signal was not sufficient. A resin gel with a stronger affinity for methyl uptake and minimal inorganic uptake was needed. Using mercury speciation chemistry and the concept of amalgamation, a method was developed to remove the diffusing inorganic mercury while allowing the methylmercury to diffuse into the resin gel. Additional filter membranes were obtained from excess Type 3 probes to create an additional filter layer underneath the present one. The underlying additional filter was conditioned with colloidal gold (3 nm) by soaking it in about 20 ppm of solution (Figure 3) and allowing the filter to dry by placing it in an oven at 95°C for about 3 hours.

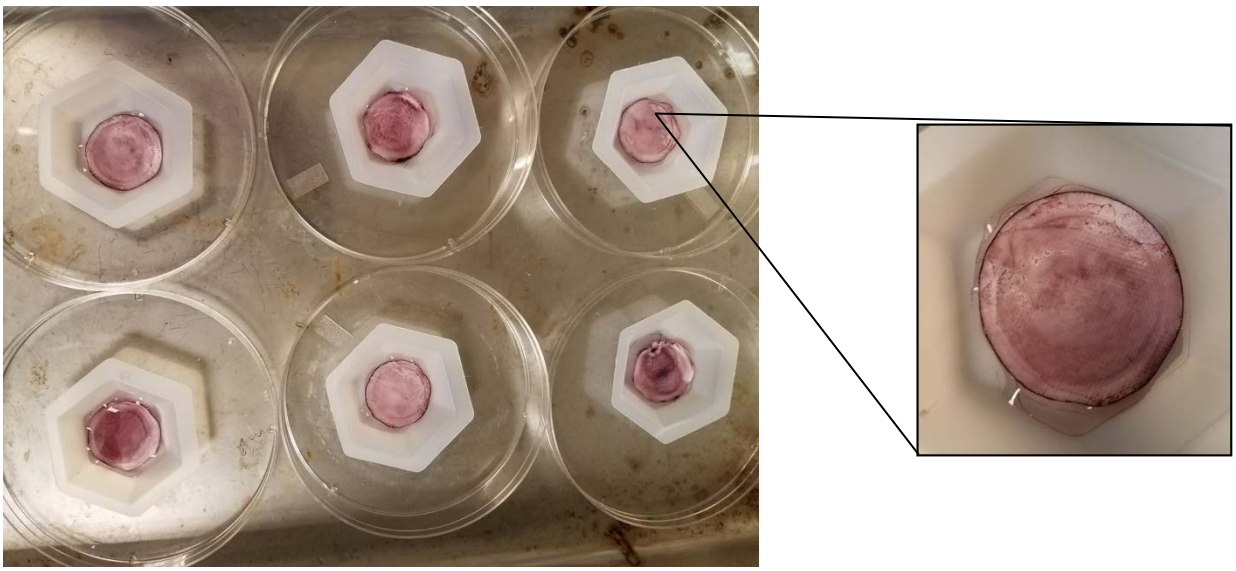

Figure 3. Filter membranes being treated with colloidal gold.

Stannous chloride powder was then prepared and finely ground using a mortar and pestle. About 0.1 g of the powder was placed in between the two filters to encourage amalgamation between the mercury and colloidal gold before the immersion solution met the diffusion layer. This setup is shown in Figure 4 and the resulting DGT in Figure 5. The DGT's were then deployed and retrieved using the same methods as in campaigns 1 and 2.

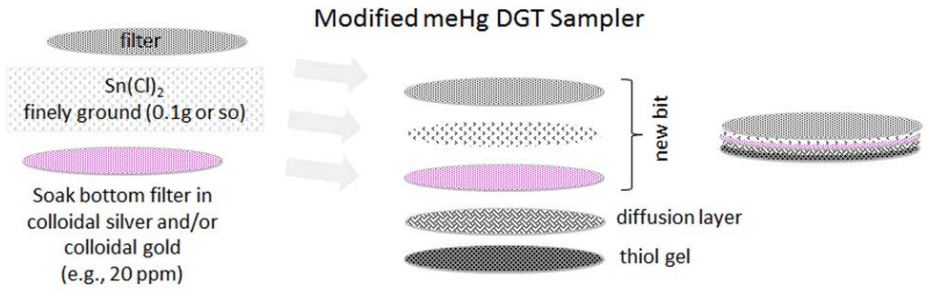

Figure 4. Schematic and exploded view of a reactive DGT probe.

Figure 5. Reactive DGT probe using $SnCl_2$ reductant without top filter and cap.

Copper Metal

The data received from campaign 3 suggested that there was an increase in MeHg uptake and a decrease in InHg uptake as a result of using colloidal gold and stannous chloride. However, the stannous chloride created more precipitate then desired and could have had a possible negative effect on the MeHg uptake efficiency. This led to the development of campaign 4. To prevent precipitate from forming, 0.16 g of copper metal powder was placed on top of each treated filter, instead of stannous chloride. The filter membrane was conditioned using the same procedure as in campaign 3 and is shown Figure 6.

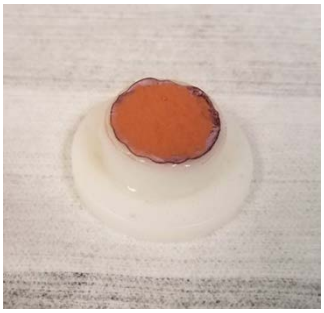

Figure 6. Reactive DGT probe using Cu (0) reductant without top filter and cap.

Theory of the Reactive DGT

The principle behind the development of a reactive DGT is to create a DGT that can differentiate mercury species using resin gels that are commercially available. The new reactive DGT

incorporates targeted chemical reactions to differentiate key mercury species. The reactive DGTs were manipulated to selectively measure either methylmercury by manipulating inorganic mercury behavior through the combined processes of chemical reduction and amalgamation. The methylmercury rDGT removes inorganic mercury using these reactions along the diffusive path – allowing methylmercury to freely move to the standard thiol based resin gel (Figure 7). For mercury speciation, the rDGT provides a quick, low cost alternative to complicated, time consuming and expensive laboratory methods currently used.

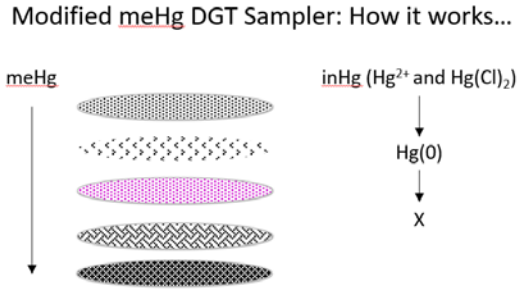

Figure 7. Selective uptake of MeHg in reactive DGT.

An additional filter membrane is added to the front of the DGT and a reductant (tin(II) chloride or Cu(0)) is added in the space created. The reductant converts incoming inorganic ionic mercury to elemental. Elemental mercury will then adhere to the colloidal gold on the underlying treated filter through amalgamation, stopping the elemental mercury from moving into the diffusion layer.

4. RESULTS AND ANALYSIS

Campaigns 1 and 2 supported the initial hypothesis; more MeHg was collected by the hydrophobic DGT (Probe Type 2) in comparison to the uptake of InHg. However, the differential uptake between the two species was not significant and there was an observable limited collection efficiency with Probe Type 2 in comparison with Probe Type 1 and Probe Type 4. These results would not support the practical application or development of a Type 2 based MeHg DGT. Probe Types 1 and 4 collected significantly higher amounts of MeHg and InHg but the differential uptake between the two species was also not significant for practical application. The data from campaigns 1 and 2 also showed a decrease in collection efficiency of Probe Type 4, indicating that the resins lose their uptake efficiency over time. Type 3 data was omitted because background levels in the Lumex were out of specification due to deposits collecting on the cell windows.

Campaign 3 supported the second hypothesis and that the principle behind the reactive DGT (rDGT) is effective. The differential uptake between the species was significantly larger in Probe Type 5 and Probe Type 6 in comparison to the probes in campaign 1 and 2. The amount of MeHg collected in Probe Type 5 was almost equivalent to Probe Type 1. Probe Type 6 collected less MeHg then Probe Type 1 and Probe Type 4; this may be due to the precipitate, tin oxide (SnO_2), produced by the $SnCl_2$ reductant diffusing out into the immersion solution.

Campaign 4 showed a significant improvement in the differential uptake between MeHg and InHg with a change in reductant from $SnCl_2$ to Cu(0). The reactive copper DGT, Probe Type 7, accumulated an average of 30.83 ng of methylmercury while Probe Type 6 had an average mercury accumulation of 22.54 ng of methylmercury. It was observed that in utilizing Cu(0) as a reductant, no precipitation or diffusion of copper was observed in the immersion solution, which could be an indication of better performance. To ensure rDGT performance, further testing is needed. In comparing all probes, Probe Types 1 and 5 had the largest MeHg uptake, while Probe Types 5 and 6 showed the greatest potential for practical applications of a reactive DGT that will selectively uptake only MeHg.

Table 1. Overall Results for DGT Testing

Probe Type	Description	M (ng)	C_i (ng/cm^3)	C_f (ng/cm^3)	AVE. C (ng/cm^3)	A (cm^2)	t (sec)	Δg (cm)	D (cm^2/sec)
LSNB Standard DGT for total mercury (practice run)	Control LSNB - A	0	n/a	n/a	n/a	3.14	0	0.094	n/a
	Control LSNB - B								n/a
	InHg LSNB -A	8.640			1.1439		86400		2.62E-06
	InHg LSNB - B	17.618					172800		2.67E-06
	InHg LSNB - C	41.853					406800		2.69E-06
LSNB$_{new}$ Standard DGT for total mercury in water	Control LSNBn - A	4.404	0.02	0.02	0.02	3.14	172800	0.094	n/a
	Control LSNBn - B	4.620							n/a
	InHg LSNBn - A	36.012	1.25	1.19	1.22				5.11E-06
	InHg LSNBn - B	31.260							4.439E-06
	MeHg LSNBn - A	49.548	1.33	1.32	1.325				6.4784E-06
	MeHg LSNBn - B	41.052							5.3675E-06
	MixHg LSNBn - A	30.828	1.29	1.24	1.265				4.2219E-06
	MixHg LSNBn - B	37.092							5.0798E-06
LSND Standar DGT for antibiotics in water	Control LSND - A	-5.568	0.02	0.02	0.02	3.14	172800	0.094	n/a
	Control LSND - B	5.628							n/a
	LSND InHg- A	3.828	0.557	0.524	0.541				1.2258E-06
	LSND InHg - B	2.460							7.8776E-07
	LSND MeHg - A	12.252	1.31	1.22	1.265				1.6779E-06
	LSND MeHg - B	11.100							1.5202E-06
	LSND Mix Hg - A	7.644	1.07	1.02	1.045				1.2672E-06
	LSND Mix Hg - B	17.364							2.8786E-06
LSNB$_{old}$ Standard DGT for total mercury in water	Control LSNBo - A	-2.364	0.02	0.02	0.02	3.14	172800	0.094	n/a
	Control LSNBo - B	-3.7176							n/a
	InHg LNSBo - A	22.692	1.1	0.496	0.798				4.9263E-06
	InHg LNSBo - B	15.060							3.2695E-06

	MeHg LNSBo - A	43.932	1.37	1.28	1.325				5.7441E-06
	MeHg LNSBo - B	29.892							3.9084E-06
	MixHg LNSBo - A	21.540	1.15	0.915	1.0325				3.6142E-06
	MixHg LNSBo - B	29.388							4.931E-06
$LSNB_{SnCl2}$ reactive rDGT with tin(II)/Au reduction and amaglamation	Control LSNBmod - A	-0.035	0.02	0.02	0.02	3.14	241200	0.108	n/a
	Control LSNBmod - B	-0.860							n/a
	InHg LSNBmod - A	-0.860	1.06	0.04	0.5495				-2.232E-07
	InHg LSNBmod - B	-2.735							-7.098E-07
	MeHg LSNBmod - A	15.040	1.39	0.95	1.17025				1.8327E-06
	MeHg LSNBmod - B	30.040							3.6605E-06
$LSNB_{2F}$ Standard DGT for total mercury in water w/ added filter	Control LSNB2F - A	-1.910	0.02	0.02	0.02	3.14	241200	0.108	n/a
	Control LSNB2F - B	-3.935							n/a
	InHg LSNB2F - A	16.765	1.41	0.595	1.0025				2.3847E-06
	InHg LSNB2F - B	12.190							1.7339E-06
	MeHg LSNB2F - A	51.190	1.35	1.23	1.29				5.6586E-06
	MeHg LSNB2F - B	45.265							5.0037E-06
$LSNB_{Cu}$ reactive rDGT with Cu(0)/Au reduction and amaglamation	Control LNSBcu - A	0.052	0.02	0.02	0.02	3.14	237600	0.123	n/a
	Control LNSBcu - B	-0.388							n/a
	InHg LNSBcu - A	-0.278	1.23	0.581	0.9055				-5.062E-08
	InHg LNSBcu - B	-0.146							-2.658E-08
	MeHg LNSBcu - A	37.100	1.36	1.23	1.295				4.7232E-06
	MeHg LNSBcu - B	24.560							3.1267E-06

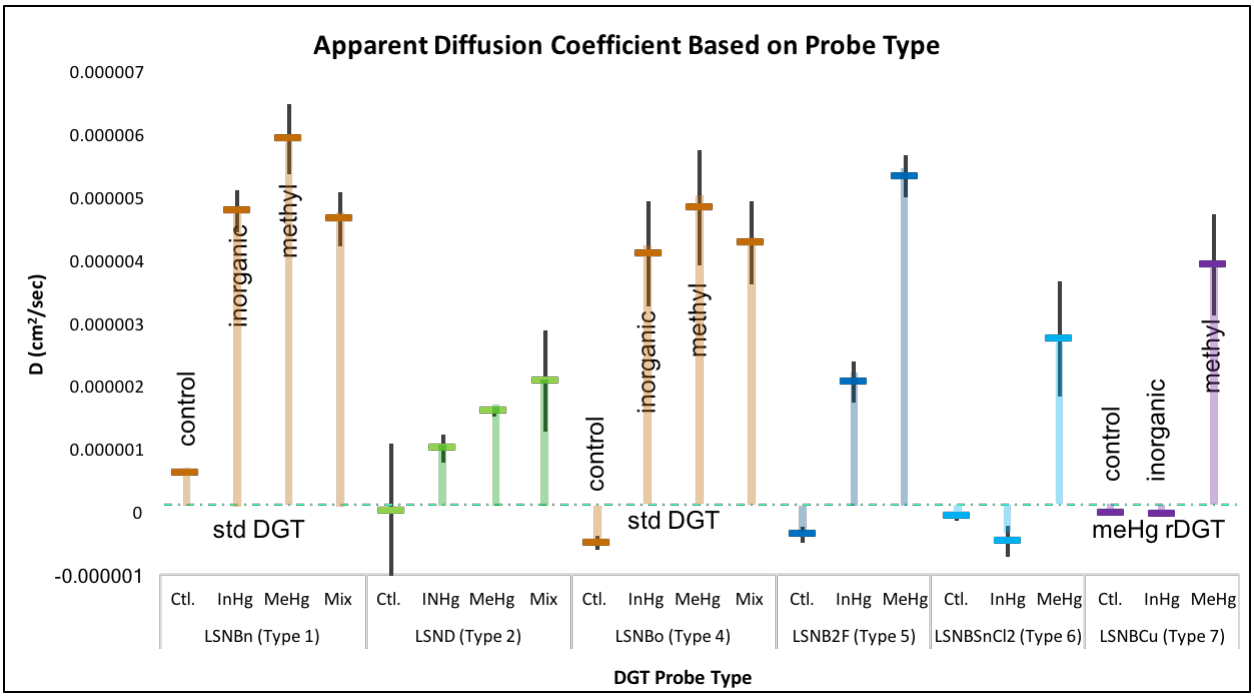

Figure 8. Differential uptake comparison of all DGT probes tested.

5. CONCLUSION

The purpose of this study was to identify a DGT probe that could be used to selectively measure methylmercury as well as develop a protocol to measure total and organic mercury via in-situ sampling in natural waters using DGT probes. This was done by running multiple campaigns that tested 3 different resins and manipulated the uptake of mercury species by modifying the DGT layers. The modified, reactive DGTs showed the greatest potential for being used to selectively measure methylmercury.

The new reactive DGT, in theory, has more biological relevance than the standard DGTs currently used. Standard DGTs are used to report the total mercury content in a biological sample which includes both organic and inorganic species. However, the bioaccumulative presence of mercury throughout the food chain is derived by methylmercury. Thus, reporting methylmercury content in the environment would enhance risks assessment of human and environmental health, and advance the ability to monitor, understand and mitigate mercury in contaminated streams, rivers, and lakes. Figure 9 demonstrates how the new reactive DGT is more reflective of how mercury speciation occurs in the environment and biological uptake.

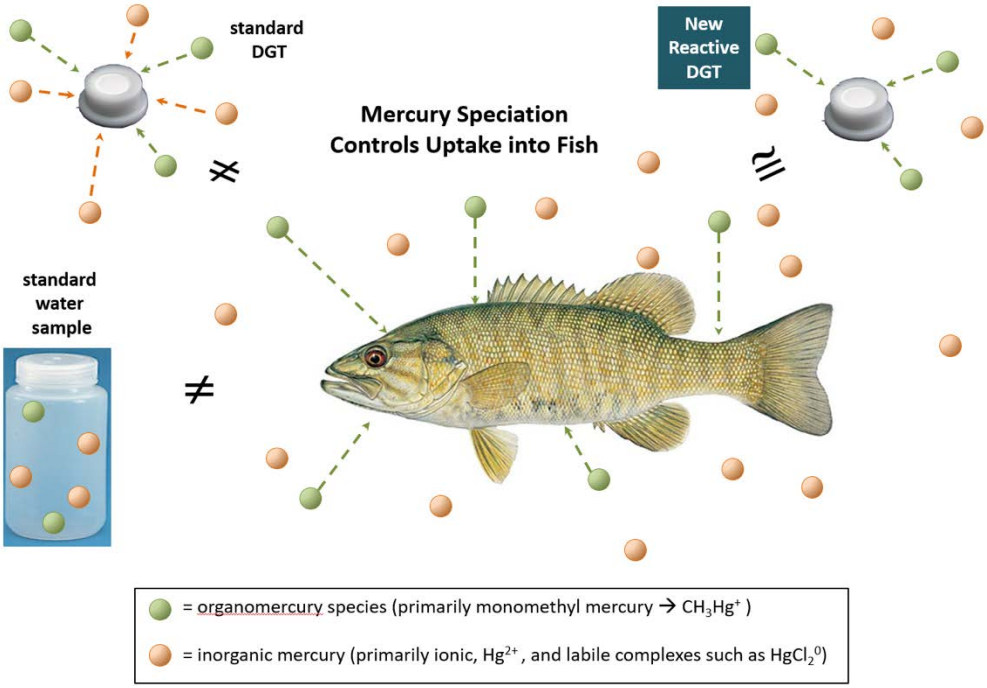

Figure 9. Biological relevance of a standard DGT and reactive DGT.

6. FUTURE WORK

Future studies on using DGTs for mercury speciation could further explore identifying an optimal reductant [such as Zn (0) or Cu (0)] for reactive DGT to measure only methylmercury. Examination of alternative configurations of the reactive DGT could also be studied since only two configurations were looked at in this study. Follow up experiments should also be performed to test the reactive DGT shelf life, effective deployment time, DOC, optimal pH, varying Hg concentration, and speciation correlation to fish uptake. Further assessment of measuring mercury with DGT technology as a low cost rapid assessment monitoring technique should also be done. SRS will be able to use this study for in-situ deployment onsite to rapidly assess water for methylmercury. The reactive DGT can be used to conduct field tests using reactive DGTs in different environments (FIU, Oak Ridge, Savannah River) to determine how they perform under an array of conditions.

7. REFERENCES

Clarisse, Olivier and Holger Hintelmann, 2006. Measurements of Dissolved Methymercury in Natural Waters Using Diffusion Gradients in Thin Film (DGT). Journal of Environmental Monitoring. www.rsc.org/jem. Pgs 1242-1247.

Zhang, Hao, 2003. DGT-for Measurements in Water, Soils, and Sediments. DGT Research Ltd.

Docekalova, H., and P. Divis, 2004. Application of Diffusion Gradient in Thin Film (DGT) to Measurement of Mercury in Aquatic Systems. Science Direct. ELSEVIER.

Fernandez-Gomez, C., B. Dimock, H. Hintelmann, and S. Diez, 2011. Development of the DGT Technique for Hg Measurements in Water: Comparison of Three Different Types of Samples in Laboratory Assays. Chemosphere, ELSEVEIR.

Paller, M.H., and James W. Littrell, 2007. Long Term Changes in Mercury Concentration in Fish From the Middle Savannah River. Science Direct. ELSEVIER.

Panichev, N.A., S.E. Panicheva, 2015. Influence of Different Cooking Procedure on the Hg Concentration in Fish. Fisheriessciences.com. Pg 64.

Pelcova, Pavlina, Hana Docekalova, and Andrea Kleckerova, 2013. Development of the Diffusive Gradient in Thin Film Technique for the Measurement of Labile Mercury Species in Waters. Analytica Chimica Acta. ELSEVIER.

APPENDIX A.

In developing a protocol for monitoring methylmercury through diffusive gradients in thin-films, a trial run was conducted utilizing the DGT probe LSNB. This was done by taking three DGT and submersing them into a 1200 ng Hg solution for 24, 48, 113 hours. Data pertaining to the trial run was normalized using average data from other LSNB DGT probe experiments conducted. To normalize the data, the following calculations were performed and charts were created.

$C_{avg,1}$

$\Delta M_1 = M_1\text{-}0 = \text{average}(C_0, C_{t1}) = \text{average (intercept, } C_{t1})$

$\Delta M_2 = M_2\text{-}M_1 = \text{average}(C_{t1}, C_{t2})$

$\Delta M_3 = M_3\text{-}M_2 = \text{average}(C_{t2}, C_{t3})$

raw data

$M_1 = \Delta M_1$

$M_2 = M_1 + \Delta M_2$

$M_3 = M_2 + \Delta M_3$

normalized data

$M_{1,n} = \Delta M_1$

$M_{2.n} = M_1 + (C_{avg,1} / C_{avg,2})(\Delta M_2)$

$M_{3,n} = M_2 + (C_{avg,1} / C_{avg,3})(\Delta M_3)$

Table 2. LSNB DGT Exposed to 1200 ng Inorganic Mercury for Set Intervals of Time

LSNB DGT Exposed to 1200ng Inorganic Mercury										
No	**Description**	**Average Area**	**M, Mass Mercury collected**	**Time before Harvested (hours)**	**Time Harvested (sec)**	**Actual Water**	**AVE. Water Period**	**ΔM**	**M, raw data**	**M_x, Normalized data**
8	inHg DGT Control initial	5630	3.661	0	0	1.2735	0	0	0	0
9	inHg DGT 1200A	8330	12.301	24	86400	1.0143	1.144	8.64	8.64	8.640
10	inHG DGT 1200B	10500	19.245	48	172800	0.7551	0.885	6.94	15.58	17.618
11	inHG DGT 1200C	13400	28.525	113	406800	0.0531	0.404	9.28	24.86	41.853
8	inHg DGT Control final	5630	3.661	113	406800					

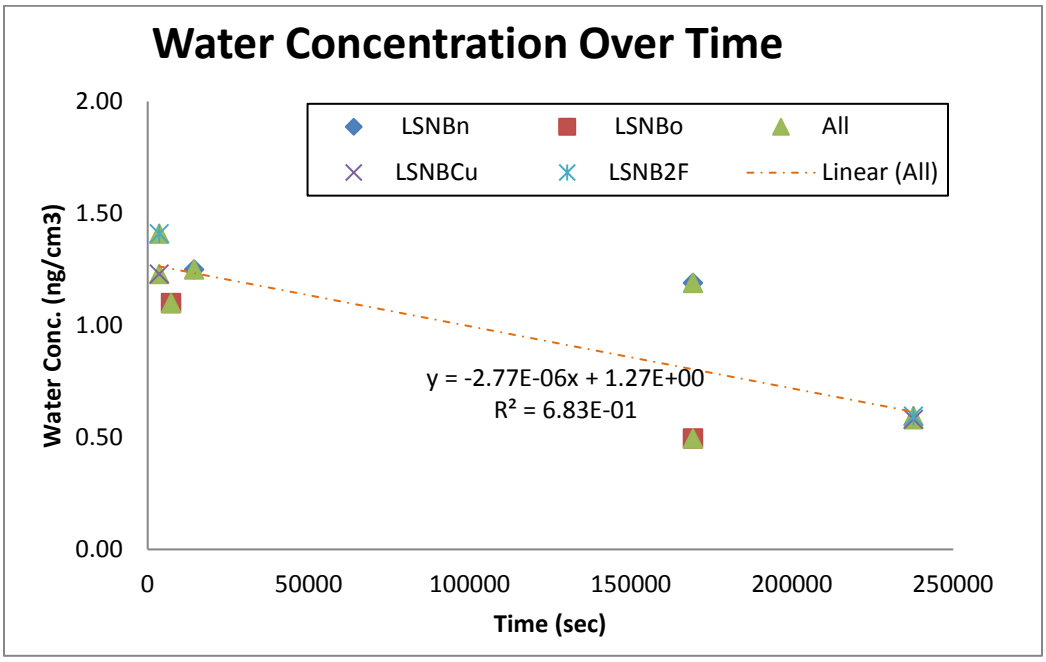

Figure 10. Mercury concentration in water samples.

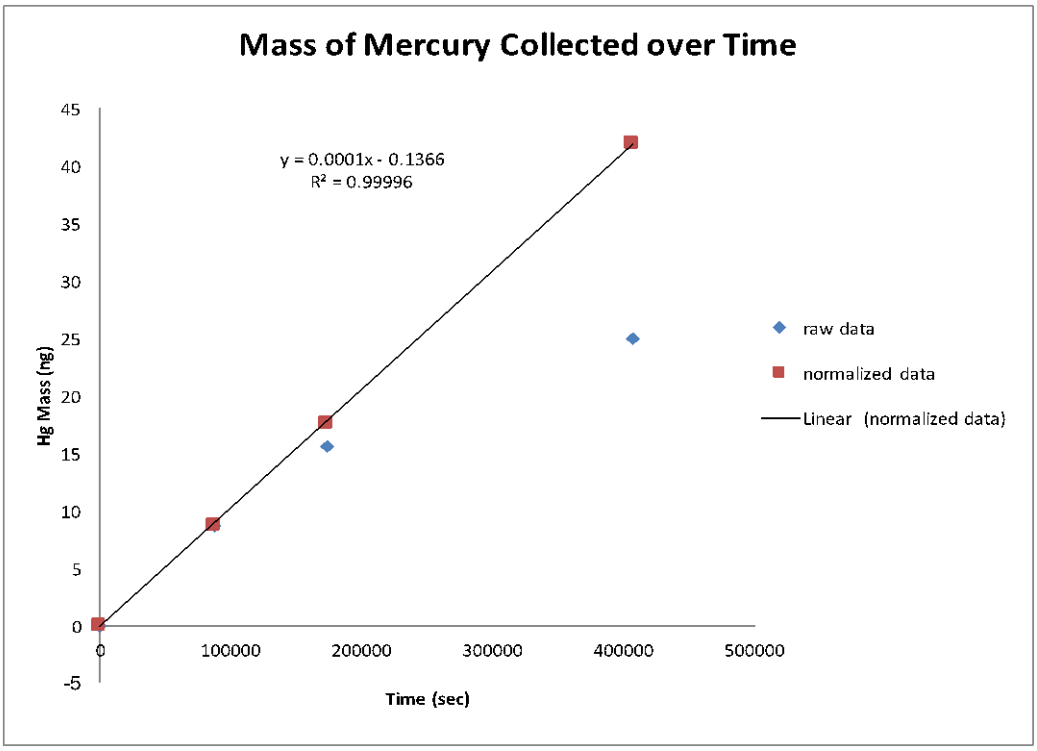

Figure 11. Mass of mercury collected over time obtained from preliminary test utilizing LSNB.

APPENDIX B.

Table 3: Water Sample Concentration

Water Samples				
Sample Id	Analyte	Result (ng/cm^3)	MDL (ng/cm^3)	AVE. Water Conc (ng/cm^3)
Control LSNBCu H2O	MERCURY	0.02	0.02	0.020
InHg 1200ng LSBNCu H2O 1	MERCURY	1.23	0.02	0.906
InHg 1200ng LSBNCu H2O 2	MERCURY	0.581	0.02	
MeHg 1200ng LSBNCu H2O 1	MERCURY	1.36	0.02	1.295
MeHg 1200ng LSBNCu H2O 2	MERCURY	1.23	0.02	
Control LSNL2F H2O	MERCURY	0.02	0.02	0.020
InHg 1200ng LSNB2F H2O 1	MERCURY	1.41	0.02	1.003
InHg 1200ng LSNB2F H2O 2	MERCURY	0.595	0.02	
MeHg 1200ng LSNB2F H2O 1	MERCURY	1.35	0.02	1.290
MeHg 1200ng LSNB2F H2O 2	MERCURY	1.23	0.02	
Control LSNBmod H2O	MERCURY	0.02	0.02	0.020
InHg 1200ng LSNBmod H2O 1	MERCURY	1.06	0.02	0.379
InHg 1200ng LSNBmod H2O 2	MERCURY	0.058	0.02	
InHg 1200ng LSNBmod H2O 1F	MERCURY	0.02	0.02	
MeHg 1200ng LSNBmod H2O 1	MERCURY	1.39	0.02	1.097
MeHg 1200ng LSNBmod H2O 2	MERCURY	0.966	0.02	
MeHg 1200ng LSNBmod H2O 1F	MERCURY	0.935	0.02	
ControlHg1200-LSNBn/D-H20	MERCURY	0.02	0.02	1.088
MeHg1200-LSNBn-H20-1	MERCURY	1.33	0.02	1.325
MeHg1200-LSNBn-H20-2	MERCURY	1.32	0.02	
InHg1200-LSNBn-H20-1	MERCURY	1.25	0.02	1.220
InHg1200-LSNBn-H20-2	MERCURY	1.19	0.02	
MixHg1200-LSNBn-H20-1	MERCURY	1.29	0.02	1.265
MixHg1200-LSNBn-H20-2	MERCURY	1.24	0.02	
MeHg1200-LSND-H20-1	MERCURY	1.31	0.02	1.265
MeHg1200-LSND-H20-2	MERCURY	1.22	0.02	
InHg1200-LSND-H20-1	MERCURY	0.557	0.02	0.541
InHg1200-LSND-H20-2	MERCURY	0.524	0.02	
MixHg1200-LSND-H20-1	MERCURY	1.07	0.02	1.045
MixHg1200-LSND-H20-2	MERCURY	1.02	0.02	
ControlHg1200-LSNBo/C-H20	MERCURY	0.02	0.02	1.062
MeHg1200-LSNBo-H20-1	MERCURY	1.37	0.02	1.325
MeHg1200-LSNBo-H20-2	MERCURY	1.28	0.02	
InHg1200-LSNBo-H20-1	MERCURY	1.1	0.02	0.798
InHg1200-LSNBo-H20-2	MERCURY	0.496	0.02	
MixHg1200-LSNBo-H20-1	MERCURY	1.15	0.02	1.033
MixHg1200-LSNBo-H20-2	MERCURY	0.915	0.02	

Table 4: DGT Raw Data

Description	M (ng)	AVE.M/AVE. water conc. ($cm^{3)}$
LNSBcu control - A	0.052	-0.153
LNSBcu control - B	-0.388	
LNSBcu InHg - A	-0.278	-0.234
LNSBcu InHg - B	-0.146	
LNSBcu MeHg - A	37.100	23.807
LNSBcu MeHg - B	24.560	
LSNB2F Control - A	-1.910	-2.550
LSNB2F Control - B	-3.935	
LSNB2F InHg - A	16.765	14.405
LSNB2F InHg - B	12.190	
LSNB2F MeHg - A	51.190	37.386
LSNB2F MeHg - B	45.265	
LSNBmod Control - A	-0.035	-0.606
LSNBmod Control - B	-0.860	
LSNBmod InHg - A	-0.860	-4.739
LSNBmod InHg - B	-2.735	
LSNBmod MeHg - A	15.040	20.547
LSNBmod MeHg - B	30.040	
Control LSNBn - A	4.404	3.546
Control LSNBn - B	4.620	
LSNBn InHg - A	36.012	27.570
LSNBn InHg - B	31.260	
LSNBn MeHg - A	49.548	34.189
LSNBn MeHg - B	41.052	
LSNBn Mix Hg- A	30.828	26.846
LSNBn Mix Hg - B	37.092	
Control LSND - A	-5.568	0.033
Control LSND - B	5.628	
LSND InHg- A	3.828	5.817
LSND InHg - B	2.460	
LSND MeHg - A	12.252	9.230
LSND MeHg - B	11.100	
LSND Mix Hg - A	7.644	11.966
LSND Mix Hg - B	17.364	
Control LSNBo - A	-2.364	-2.865
Control LSNBo - B	-3.718	
LNSBo InHg - A	22.692	23.654
LNSBo InHg - B	15.060	
LNSBo MeHg - A	43.932	27.858
LNSBo MeHg- B	29.892	
LNSBo Mix Hg- A	21.540	24.662
LNSBo Mix Hg - B	29.388	