FINAL REPORT

Proof-of-Concept for the *in situ* Toxicity Identification Evaluation (iTIE) Technology for Assessing Contaminated Sediments, Remediation Success, Recontamination, and Source Identification

SERDP Project ER18-1181

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Chemical mixtures, Mixture toxicity, Causal linkages, Weight-of-evidence, Restoration success, Remediation success, Biological fractionation, Perfluorooctanesulfonic acid (PFOS), Per- and Polyfluoroalkyl Substances (PFAS)

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List of Acronyms

DoD	Department of Defense
ESTCP	Environmental Security Technology Certification Program
IPR	In-Progress Review
iTIE	*In situ* Toxicity Identification Evaluation
ITIES	*In situ* Toxicity Identification Evaluation System
IToDS	*In situ* Toxicity Diagnostic System
PFAS	Per- and Polyfluoroalkyl Substances
PFOS	Perfluorooctanesulfonic acid
SEED	SERDP Exploratory Development
SON	Statement of Need
TIE	Toxicity Identification Evaluation
WoE	Weight-of-Evidence

Keywords

Chemical mixtures, Mixture toxicity, Causal linkages, Weight-of-evidence, Restoration success, Remediation success, Biological fractionation, Perfluorooctanesulfonic acid (PFOS), Per- and Polyfluoroalkyl Substances (PFAS)

Acknowledgment

The iTIE device was originally developed by G.A. Burton, Jr with J.F. Nordstrom. A. Steigmeyer (2015) and K. Meyer (2016) completed theses at the University of Michigan (USA) optimizing the iTIE device with support from the University of Nanjing, China. SeaView (MI, USA) completed the refinement of the electronics and pumping device. Strategic Environmental Research and Development Program (SERDP, Contract No. W912HQ18C0019, Project No. ER18-C4-1181) and Water Research Foundation (WERF) grants along with LimnoTech and Burton in-house contributions have supported the development of the iTIE (now called IToDS). A number of students and staff supported deployments.

Abstract

Introduction and Objectives: This SEED project addressed critical Department of Defense (DoD) issues regarding the need for effective monitoring tools that provide certainty in the decision-making process with regards to critical risk determination components, such as causality, bioavailability, source identification, and fate across ecosystem compartments. The *in situ* toxicity identification evaluation system (iTIES) can address all of these critical risk determinants in a cost-effective manner. It is a biological, fractionation protocol that systematically identifies chemical classes causing toxicity in overlying water, porewater, and outfalls (i.e., industrial/municipal point source and stormwater). The system separates chemical classes of contaminants of concern frequently linked to adverse biological effects (i.e., various types of organics, metals, ammonia) at DoD sites. The overall objective of the proposed project is the proof-of-concept of an accurate field methodology for *in situ* assessment that links chemical class exposures to effects, allowing for more cost-effective monitoring and remediation decisions.

Technical Approach: The iTIES prototype 3 is a robust deployable system that allows for consistent and sensitive adjustments to pumping rates of ambient waters through the diagnostic array of resin treatments. The current battery of resins separates the following potential toxicants: ammonia, problematic heavy metals (Ag, Cd, Cu, Ni, Pb, Zn), and organics of various characteristics, including per- and polyfluoroalkyl substances (PFAS).

Results: A proof-of-concept was established for the iTIES. The iTIES appears to be more sensitive at detecting ambient toxicity than the traditional laboratory-based TIE and requires fewer resources to conduct an experiment. It provides a unique diagnostic tool for use in a tiered risk assessment. Its applications to a host of critical DoD concerns and SONs suggest it should become a standard diagnostic assessment technology at chemically contaminated sites. An evaluation was made of the resources required for conducting the traditional US Environmental Protection Agency Phase 1 TIE and the iTIES. Because costs per hour vary by organization, the comparisons were based on staff time required for each task. The differences are quite dramatic, with the iTIES requiring 47% less time (67 fewer hours). The iTIE has been shown to be more sensitive at detecting toxicity than the laboratory-based TIE. If the laboratory TIE has a higher potential for false negative results, then the diagnostic ability of this approach is poor and may result in poor decision-making regarding site management.

Benefits: From a strategic assessment approach, the iTIE should be viewed as a Tier 2 or 3 level approach for incorporation into a smart weight-of-evidence study. It is a diagnostic tool to be used once Tier 1 assessments suggest chemical toxicity may be a concern. Because most sites contain a plethora of chemicals which may be contributing to toxicity, the iTIE can direct the site manager to focus on those chemicals of greatest ecological concern. In addition, the iTIE can assist in source identification of toxic chemicals whether associated with sediments, caps, ambient waters, stormwaters, or outfalls. Before the iTIES can become a standard diagnostic technology, it requires additional development. Additional research should include:

- Refining the porewater sampling option – verify sediment porewater sampling zone versus surface water infiltration and gentle aeration of toxicity chamber porewater;
- Improving deployment logistics of iTIE chambers to sediments/porewater (diver vs diverless options);

- Continuing to optimize resin selectivity for various target chemicals (e.g., perfluorooctanesulfonic acid [PFOS]);
- Assessing deployment depth limitations (optimal would be up to depths of 20 m);
- Developing an underwater pumping container option (increases versatility for where and how long deployment can occur);
- Testing of early life stage fish;
- Expanding sublethal, chronic endpoints;
- Additional field verifications (marine and freshwater).

Executive Summary

Introduction

Despite the Strategic Environmental Research and Development Program (SERDP)/Environmental Security Technology Certification Program (ESTCP) supporting the development of *in situ* tools for monitoring contaminants and characterizing environmental effects, a gap remains with respect to realistic, *in situ* tools that link measurements of specific chemical exposures with biological effects in a way that provides direct information on the chemicals responsible for the observed effects. This critical uncertainty impedes effective management decisions on whether or not sites must be remediated, which chemicals are responsible for the adverse effects, and whether or not remediation technologies are working well. Current monitoring costs are excessive and particularly ineffective in establishing causality and linkages to improved biological communities. At most sites where multiple line-of-evidence approaches are used, chemical causality is not established, and decisions are made of the basis of best professional judgment or are regulatory bright-line based. These weight-of-evidence (WoE) approaches tend to be crude, qualitative, and highly uncertain in their conclusions. Although laboratory toxicity identification evaluation (TIE) methods exist (Anderson et al 2008, 2010; Ho and Burgess 2013; Ho et al. 2002; Hunt et al., 2001, 2008; Phillips et al., 2004, 2006, 2009; US Environmental Protection Agency 1996, 2007), they suffer from the same lack of realism (being prone to sampling and manipulation artifacts) as shared by other laboratory exposure and effects measurements.

The authors of this SEED project addressed critical Department of Defense (DoD) issues regarding the need for monitoring tools effective at providing certainty in the decision-making process with regards to **critical risk determination components, such as causality, bioavailability, source identification, and fate across ecosystem compartments. The *in-situ* TIE system (iTIES) can address all of these critical risk determinants in a cost-effective manner.** It is a biological, fractionation protocol that systematically identifies chemical classes causing toxicity in overlying water, porewater, and outfalls (i.e., industrial/municipal point source and stormwater). The system separates chemical classes of contaminants of concern frequently linked to adverse biological effects (i.e., various types of organics, metals, ammonia) at DoD sites. The initial iTIE prototype technology effectively separated 3 groups of chemicals and confirmed that polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls

(PCBs) in sediments were responsible for negative biological impacts (Burton and Nordstrom 2004a, 2004b). The iTIE exposures can utilize a wide range of macroinvertebrate and fish species to allow linkage to indigenous benthic and fish populations and community indices. A second iTIE prototype recently developed consists of a single unit capable of housing an array of iTIE units (Appendix A, Figure A1; Steigmeyer et al. 2017), including a space for multiple resins that can be selective for various classes of organic compounds. Chemical confirmatory analyses (as with the US Environmental Protection Agency's [USEPA's] laboratory-based TIE Phases 2 and 3) may be conducted on the collected water and absorbent resins to establish causality by linking to biological effects. **Biological endpoints measured vary with the organism tested. Endpoints range from genomic to mortality responses**.

The iTIES should be a higher Tier (2 or 3) diagnostic component in a strategic ecological risk assessment. In a Tier 1 assessment of ecological risk, site contamination, or restoration effectiveness is conducted and if elevated chemical contaminants or impaired benthic or fish communities are found; the iTIES can be utilized to identify in a Tier 2 or 3 assessment to determine which chemical classes are driving toxicity and the relative toxicity contribution of various sources (e.g., surface waters, sediments, outfalls, stormwater). For a strategic WoE monitoring/assessment, iTIEs should be coupled with other proven and accurate monitoring tools, such as the SEA Ring technology (SERDP ER-1550/ESTCP ER-201130) and passive sampling devices (PSDs) to better link chemical exposures with adverse biological effects.

The iTIES process has 7 steps: (1) identifying the possible groups of contaminants and pathways of concern; (2) selecting appropriate absorbents, organisms, and endpoints; (3) preloading iTIEs in the laboratory or field; (4) deployment at the site (6–48 h); (5) retrieval; (6) evaluating toxicity and/or biomarker endpoints; and (7) processing the water and/or resins for chemical analyses.

At sites with a myriad of potentially toxic chemicals, it is critical that a link between the bioavailable fraction of the chemicals (not just the contaminants of concern) and those contributing most directly to toxicity is determined. This determination is possible using the iTIE, which is much less resource intensive and more sensitive than the USEPA's laboratory-based TIEs (Burton and Nordstrom 2004a, 2004b, and current project). The USEPA methods tend to produce more artifacts, due to sample collection and extensive laboratory manipulations of the samples. The USEPA methods often consist of porewater extraction by centrifugation or suction, and the addition of sorptive materials (e.g., coconut charcoal) directly to mixed sediments result in drastic alterations of redox, pH, microbial transformation rates, and most importantly – chemical bioavailability. This results in highly uncertain relationships between laboratory data and *in situ* conditions (i.e., study site realities).

The iTIES has evolved through 3 prototypes, with significant improvements with each iteration over the past 4 yr. Our latest prototype has a redesigned multi-water pump circuit replacing an air pump-based Venturi system. Prototype 2 developed 2015–2017 was tested below municipal wastewater outfalls in Denver (CO), Boise (ID), and Chicago (IL), but was plagued by the operational system for pumping ambient waters and a crude design. Multiple organic chemical absorbents have been evaluated in the past 3 yr to better separate strongly nonpolar to slightly nonpolar classes, and per- and polyfluoroalkyl substances (PFAS). These absorbents included C18 SEP Pak (Waters), activated carbon (Marineland), Oasis HLB (Waters), Oasis WAX (Waters), and Chelex (Sigma-Aldrich; Meyer 2016, Reible 2018, current project)**. The current iTIE protoype 3 (Figure 1) is robust, reliable, and easy to use in the field. This system is**

innovative, providing scientific benefits from this proof-of-concept that could improve DoD decision-making at sites with multiple contaminant issues.

Figure 1. The current *in-situ* Toxicity Identification Evaluation (iTIE) fractionation system – Prototype 3

Objectives

The overall objective of the project is the proof-of-concept of an accurate field methodology for *in situ* assessment that links chemical class exposures to effects, allowing for more cost-effective monitoring and remediation decisions. This addresses the gap between exposure and effects and provides a new tool consistent with the efforts of SERDP/ESTCP toward the development of more direct "*innovative approaches for both monitoring and implementing in-situ remediation of contaminated aquatic sediments*" (quotes from SON). This technology will "*ultimately reduce costs associated with monitoring and treating contaminated aquatic sediments, while still being protective of the environment*". Specific objectives are "*the development of an optimal strategy to reduce monitoring costs through sampling methods*" and an "*improved understanding of the utilization of lower cost bioavailability measures as surrogates for higher trophic level sampling events*" in any depositional aquatic sediments (including caps), their overlying waters and local outfalls.

Success was measured by accomplishing the proposed Tasks 1–4, with minor modifications. We demonstrated the iTIE prototype 3 to be robust, reliable, and easy to use in marine and freshwater applications.

Technical Approach

The project consisted of 3 sequential tasks as follows: Task 1: Refine the prototype iTIE for marine sediment application; Task 2: *In situ* TIE laboratory and field deployments; and Task 3: Preliminary strategy and cost effectiveness determination.

Task 1: Refine the prototype iTIE for marine sediment application

This task involved components described in Table 1 which is copied from the funded proposal. All design issues were addressed, with the exception of having a pumping unit that could be submerged. The pumping unit (Figures 2–4, Appendices A, B, D, E) is compact and waterproof from rain, but not designed to be submerged. We determined submersion was not critical for the success of this proof-of-concept because pumping could occur on board a boat or on-shore. In the future, it may be useful to have the option of submersion; however, designing and building this option was beyond the resources provided in this SEED grant.

Table 1. Task 1: Prototype Redesign Issues to be Evaluated

Major Components	Re-Design Options
Electronic controls for pumping and aeration	Robust, low power requirements, smaller size
Pumps	Robust, low rate, adjustable, consistent across treatments, small size
Resins and sorptive materials	Increase number of chemical class specificity, reduced cross-selectivity, reasonable cost and availability
Organism chamber size	Increased size for fish larvae exposures
In-situ deployment container	Water proof, deployable in diverse habitats (deep water; ~3–15 m), withstand prop-induced currents, mount adjacent to outfalls, surface water or porewater, diverless deployment

We were instructed at the last In-Progress Review (SERDP IPR Symposium 2018) to not focus on porewater testing as part of this SEED project. It was recognized that this may require more time and resources than were available for the project. Porewaters are often anoxic (particularly in contaminated sediments), however, benthic macroinvertebrates require dissolved oxygen (albeit low for some) to live and reproduce. The first iTIE prototype gently aerated the pumped porewaters allowing oxygen to diffuse through the Venturi-based silicone tube system. This gentle introduction of oxygen allowed for a realistic exposure of benthic organisms in the iTIE exposure chamber. As noted by the USEPA in their sediment collection guidance for toxicological testing (US Environmental Protection Agency 2001), this is a realistic exposure without the associated artifacts produced from sediment dredging and sample manipulation in the laboratory. Our new prototype 3 has an adaptor for the unit base for porewater extraction, but the aeration system needs to be redesigned. This will be the focus of a future SERDP proposal, if requested.

We have been optimizing resin types since 2016 (Meyer 2016; Steigmeyer et al. 2017, Appendix A). The optimization continued in this project and will likely always be a consideration as new resins are developed for specific chemical types. The chemical classes we are targeting for differing iTIE treatments include: 1) highly nonpolar organics (e.g., PCBs, dioxin, organochlorines); 2) divalent cationic metals (e.g., Cd, Cu, Ni, Pb, Zn), 3) ammonia; 4) emerging contaminants of mixed polarity (e.g., perfluorooctanesulfonic acid [PFOS]/perfluorooctanoic acid [PFOA], polybrominated diphenyl ethers, some pesticides); and 5) organics of lower *n*-octanol/water partition coefficients (e.g., PAHs). We determined that the following resins are

useful for iTIES studies: zeolite (ammonia), chelex and activated carbon (divalent metal cations of concern), Oasis HLB and C18 SPE (nonpolar organics), and Oasis WAX (PFAS; Appendix A, Figure A3). The optimal volume of resin is 5 g using a pumping rate of 25 mL/h.

As resin technology improves, it may be possible in future projects to utilize commercially available molecular imprinted polymers designed to target specific chemicals such as pesticides, PAHs, estrogens, phenols, and bisphenol A (e.g., Affinisep.com). These products are high performance sorbents based on molecularly imprinted polymers that provide superior selectivity in comparison with standard resins. They are designed to specifically recognize one target compound or structurally related target compounds based on their shape and chemical functions. These polymers will allow the detection of low-level exposures to contaminants of emerging concern (CECs) which are common in municipal effluents and may be important at some DoD sites.

Also, in future projects, we would like to develop sublethal, chronic endpoint measures, such as is possible with oyster, urchin, and fish embryo endpoints of teratogenicity and behavioral effects. This proved to be beyond the resources and time allowed for this SEED project.

Task 2: In situ TIE laboratory and field deployments

Field testing was conducted at 3 sites. First, a preliminary Prototype 3 iTIE unit was tested in D. Rieble et al. SERDP ER-2428 at the mouth of Paleta Creek in San Diego (Southern California Coastal Water Research Project and Navy 2005; Appendix A, Figure A4). The project was described in Reible et al., 2018. This prototype was further developed prior to receiving SEED grant funds, with "bridge" support funds from LimnoTech and Dr. Burton's discretionary research account.

A comparison was made between the iTIES and a laboratory-based USEPA Phase 1 TIE. This study is described in detail in Appendix B. The test organisms used in the comparison were standard marine toxicity test methods: the rotifer *Brachionus plicatilis*, Mediterranean mussel embryos (*Mytilus galloprovincialis*), and purple sea urchin embryos (*Strongylocentrotus purpuratus*). The choice of these organisms builds on the knowledge gained from our previous SERDP project ER-1550, where we used several test organisms. These organisms are supplied locally (not culturable). Additional species used successfully in our SEA Ring include: the amphipod (*Eohaustorius estuarius*), a marine polychaete (*Neanthes arenaceodentata*), and the mysid shrimp (*Americamysis bahia*).

Access to US Navy sites with known contamination (mouth of Chollas and Paleta Creeks) was prohibited during our field testing, so we created a copper contaminated mesocosm that was deployed off the SPAWAR Systems Center Pacific pier. This deployment used the final version of prototype 3 and was successful, and as discussed above, compared to a simultaneously conducted Phase 1 TIE in the Space and Naval Warfare Systems Command (San Diego, CA, USA) laboratory (Appendix B, Figure B1). Three commercially available resins (Chelex, C18 SPE, and Oasis HLB) were tested in the iTIE units. Resins were compared to a control containing glass wool (no removal). The laboratory TIE was conducted using a series of 5-stage manipulations: baseline toxicity study, filtration, aeration, metal determination (ethylenediaminetetraacetic acid [EDTA]), and organic compounds extraction (C18 SPE cartridge). After each chemical fractionation, test organisms were assessed for their acute endpoints.

Freshwater studies consisted of 2 components, with the first being an addendum to the proposed study plan. Per- and polyfluoroalkyl substance contamination has become an important topic for the DoD and is a highly publicized issue being addressed by the Michigan Department of Environmental Quality (MDEQ). The MDEQ has discovered widespread contamination in the State's streams, and the Clinton River near Detroit is one of the hotspots (PFOS > 600 ng/L). Consequently, we studied the optimal resins for use in the iTIE for isolating PFAS compounds. Discussions with Professor Chris Higgins (Colorado School of Mines) led us to using the resins activated carbon (AC; Marineland), Oasis HLB (Waters), Oasis WAX (Waters), and glass wool (Sigma-Aldrich). In addition, a field deployment of the iTIES was conducted on the Clinton River.

Task 3: Preliminary strategy and cost effectiveness determination

The final task addressed one of the important SON objectives (and support the proof-of-concept rationale for follow-up SERDP funding) and a cost-benefit analysis of the iTIE for potential users. The resources (materials and labor) required for conducting the iTIE at Naval Base San Diego (NBSD) and the University of Michigan were tracked and assessed for the field deployments. Resource costs considered the species and endpoints selected, typical boat and diver time (for future deep-water deployments) based on past experience at SSC Pacific, chemical-specific resins, personnel hours (not dollars, because labor costs vary between organizations), and chemical analyses (commercial cost rate). Similar resource requirements for the laboratory TIE (including sediment sampling) and standard laboratory toxicity testing were determined for comparison purposes.

We determined qualitatively the benefits of the approaches, with regards to the incorporation of this technology into an integrated WoE assessment strategy, its likelihood for regulatory acceptance, and the usefulness of the iTIE data in decision-making. **Uncertainty components addressed included technology sensitivity, discriminatory ability, and precision and accuracy** compared with traditional testing. The strengths and limitations of this technology and other more commonly used methods (including USEPA's TIE method) were compared. In addition, a simple, strategic, decision-making framework was developed to assist in determining which assessment tools should be used at contaminated and remediated sediment sites, incorporating the iTIE and other approaches, such as the SEA Ring, PSDs, and fish and benthic community surveys.

Results and Discussion

Task 1: Refine the prototype iTIE for marine sediment application

All components of Task 1 (Table 1) were successfully completed, with the exception of the underwater application and porewater modifications (discussed above). A critical shortcoming of previous iTIE systems has been the pumping system. To this end we were able to obtain Quad Peristaltic pump units (Welco) and the accompanying Pelican cases that were optimized and tested by SeaView Systems, Dexter, MI. The compact and portable unit is a robust design and easily adjusted to allow for a range of pumping rates. We determined in past studies that 25 mL/h is optimal because this will not exceed the capacity of the resins to sorb target compounds and does not create an undue "cone of depression" when extracting porewaters. The low power requirements allow a battery pack to pump for 24 hr, as required for this iTIE design.

The iTIE pump unit is used to house the electronic controller along with other components which connect to the primary iTIE housing array. The unit was modified to consist of a waterproof

housing containing a programmable circuit board, pumping units, and rechargeable battery (Figure 2, Appendix D). The iTIE pump units are comprised of 4 synchronous peristaltic pumps that are housed in a Pelican 1400 Protector case along with control electronics. Currently, 4 iTIE units have been manufactured, allowing for 16 iTIE units to be tested. Pumping capacity is from zero to 500 mL h^{-1}. The pumps are controlled by a microprocessor that allows for precise control of the pumping rate and stores the calibration. An integrated digital display shows the approximate volume of water pumped by 1 unit.

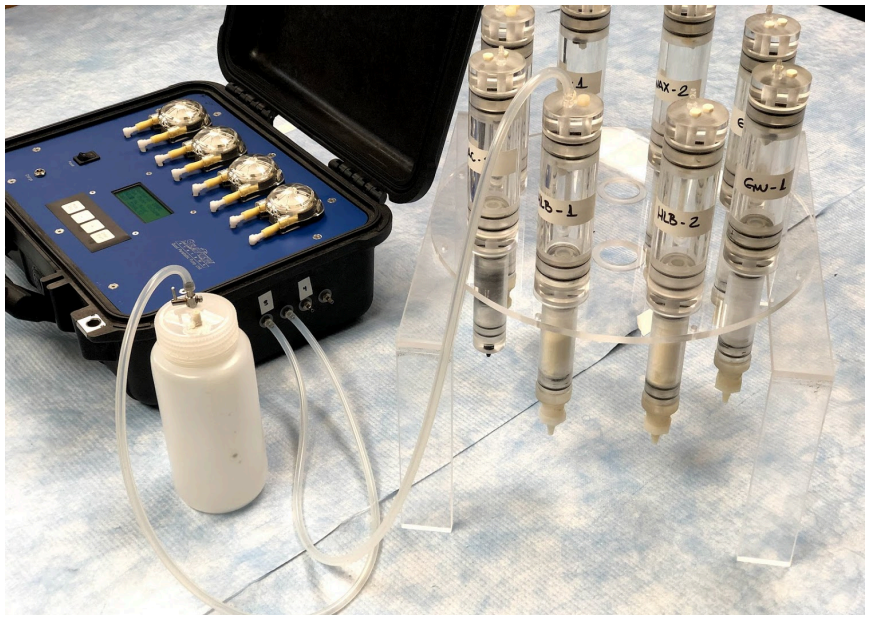

Figure 2. The in-situ Toxicity Identification Evaluation (iTIE) pump unit.

Numerous tests have been conducted in the laboratory to determine optimal pumping rates with several resin types (Figure 3). Both porewater and surface water TIE units have been tested under variable pumping rates. Typically, pump rates must be set high to overcome water pressure issues within each iTIE unit (200–300 mL h^{-1}) and then lowered after approximately 1 h.

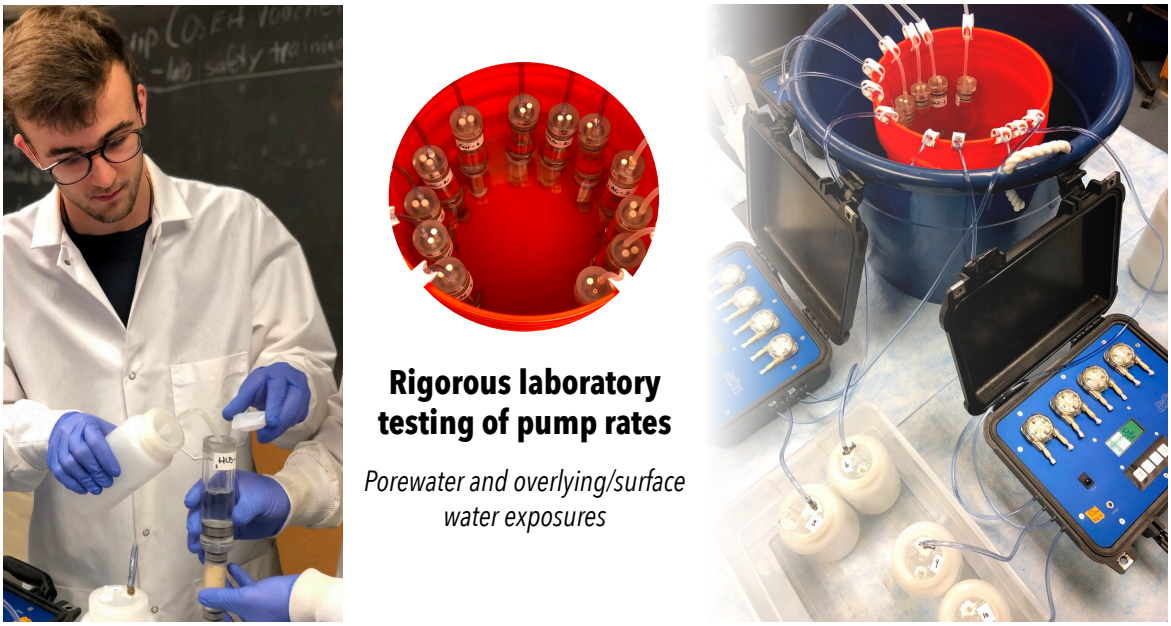

Figure 3. Laboratory testing of pump rates and resin selection for different contaminants of concern.

An iTIE deployment case was designed to create an all-in-one portable laboratory for this project (Figure 4). The case is housed in a Pelican iM2750 storm travel case and includes space for the iTIE pump unit and additional components as required for stationing the iTIE deployment case for sample collection. These include:

- Space for a single iTIE pump unit (iTIE pump unit can also be removed)
- Space for up to 100 m of tubing under the pump unit
- Legs and footpads with necessary hardware to attach to the case for water deployment which are stowed in the iTIE deployment case for transport
- Holders for 4 collection bottles and 4 iTIE water column spikes
- Tubing and barbed connectors for attaching the iTIE water column spikes

A larger size organism chamber was also designed for the exposure of early-life stage fish (Appendix D, Figure D1). No permit was acquired to allow for fish testing, so the chamber has not been field verified.

Task 2: In situ TIE laboratory and field deployments

Field results showed, as expected, that Chelex removed 93% of Cu, whereas HLB and C18 SPE removed only 6% and 16% of Cu, respectively. No rotifers were recovered from the exposure chambers in either the reference or Cu chemtainers. Urchin recovery was low in general, but likely Cu breakthrough resulted in toxicity in the Chelex treatment. Mussel larvae only survived in the Chelex treatment, showing Cu was the toxicant and was adequately removed by the metal-specific resin. In the laboratory TIE, C18 also removed Cu, in addition to EDTA. This non-target removal confounds interpretations of the type of toxicant causing toxicity. **Results show that the**

iTIE system is more sensitive and diagnostic than the laboratory TIE approach and can be used to provide an accurate profile of a given isolated environmental stressor.

Figure 4. Deployment case for easy transport and housing of iTIE parts.

For the laboratory-based TIE Phase 1, the Cu water exposures to rotifers showed EDTA and C18 treatments reduced toxicity. Aeration and 0.45µm filtration of the sample only slightly reduced some toxicity relative to the base sample. For the mussel embryo-larval development test, only the EDTA treatment had survival (97% normal development). For the urchin embryo-larval development test, results were similar with 88.5% normal development in the EDTA treatment only.

The C18 treatment reduced toxicity for the acute rotifer exposure, but did not for both chronic larval-development endpoints. The median lethal concentration (LC_{50}) for rotifers (80 µg/L [Snell and Persoone 1989; ASTM International 1998; Arnold et al. 2010]), is higher than the median effect concentration (EC_{50}) for mussels and urchin (8.0 and 14.9 µg/L, respectively; Rosen et al. 2008). This suggests that C18 was able to provide some protective effects from Cu toxicity, but not as much protection as EDTA.

In summary, mussel recovery from the *in situ* exposure was too low to be reliable. In the iTIE setup compared with the laboratory exposure, urchin recovery did appear to be adequate, although development was not as "clean" (meaning the pluteus larvae were not as well developed). This may have been due to the manipulations and physical stress placed on developing larvae when transferred from the iTIE exposure chambers into scintillation vials after 24 h of deployment. These issues have been noted for the both species in previous *in situ* exposures (such as in the SEA Ring).

Since the rotifer has a 24 h test duration, there was difficulty with hatching. In addition, there was no rotifer recovery in either the reference of Cu treatments *in situ*. The use of a more robust

organism, such as the mysid shrimp, *Americamysis bahia*, may be preferable to the use of the rotifer, or either larvae species for that matter, because mysids have an alternate 24 h acute endpoint as well, and have shown higher recovery in previous iTIE and other *in situ* deployments.

Leachate estimates of the Cu plates used in the *in situ* mesocosm resulted in an excess concentration of Cu, thus causing breakthrough in the Chelex treatment. The Chelex resin in the iTIE treatment chambers were green, suggesting it bound the high Cu concentrations and likely became saturated, thus allowing for breakthrough of Cu. As in previous comparisons (Burton and Nordstrom 2005), results suggest the iTIES results in greater exposures of contaminants, thus greater sensitivity and toxicity, than the traditional laboratory-based TIE.

This current deployment showed a successful deployment of urchins and mussel larvae in the same chamber; however, in practice, this was complicated due to the difference in test duration (i.e. 48 and 96 h test durations for mussels and urchins, respectively).

The PFOS studies provided useful information. The laboratory study (Appendix C) showed all resins removed >93% of PFOA and PFOS compared to the glass wool control (removal of 28 and 60% of PFOA and PFOS, respectively). The removal of these compounds in the glass wool control was due to a pumping pressure issue that reduced the sample volume. The analytical chemists subsequently diluted the sample to obtain adequate volume, resulting in lower PFOA/PFOS values. The loss was not due to adsorption to the glass wool. Oasis HLB was the most efficient resin to absorb PFAS from the water (96 and 98% removal for PFOA and PFOS, respectively). However, both replicates from HLB treatment had resin leakage into the exposure chamber, possibly causing toxicity to *D. magna* (survival of 50%). Survival of *D. magna* for Oasis WAX was 95%, whereas 85% of the organisms survived using AC. A follow-up field deployment at the Clinton River hotspot was recently conducted (Appendix D, Figures D2 and D3). Water chemistry results processed by each iTIE are pending.

Task 3: Preliminary strategy and cost effectiveness determination

An evaluation was made of the resources required for conducting the traditional USEPA Phase 1 TIE and the iTIES. Because costs per hour vary by organization, the comparisons were based on staff time required for each task (Table 2). Many of the components of conducting the resource analysis are comparable, such as for organism care (organisms are purchased and then cared for the day prior to testing) and test termination analyses. The primary difference is with the preparation of equipment, mobilization, test maintenance/termination, and demobilization/clean-up. The end comparison differences are quite dramatic, with the iTIES requiring 47% less time (67 fewer hours). Of course, these are estimates and may vary depending on who is conducting the testing, their level of experience, and field deployment logistics.

As noted above, our experience with the iTIE since its inception, is that it is more sensitive at detecting toxicity. This is not surprising given the many sampling and laboratory manipulations, sample handing times, and laboratory exposures based on grab samples. If a laboratory TIE has a higher potential for false negative results, then the diagnostic ability of this approach is poor and may result in poor decision-making regarding site management. The laboratory approach is likely more precise than *in situ* exposures, because field exposure tends to produce greater replicate variability. However, this can be dealt with by using multiple replicates. This shortcoming is counter-balanced by the greater accuracy showing results more reflective on *in situ* conditions (e.g., site realities).

Table 2. Resources required for the Laboratory-based TIE Phase 1 and the *in-situ* based TIE.

Executed task		Lab-based TIE		*In-situ* TIE	
		#Staff	Hours/day	#Staff	Hours/day
Organism care		1	2h/1d	1	2h/1d
Preparation of laboratory/field equipment		2	4h/2d	2	3h/1d
Mobilization/deployment	*Field*	3	8h/1d	3	4h/1d
	Laboratory	2	8h/1d	—	—
Test maintenance		2	6h/1d	1	2h/1d
Test termination		3	8h/1d	3	3h/1d
Demobilization/clean-up		2	6h/1d	2	4h/1d
Test termination analyses	*Rotifer test*	1	8h/2d	1	8h/2d
	Mysid shrimp	1	4h/1d	1	4h/1d
	Bivalve embryo	1	8h/2d	1	8h/2d
Total (h)		**142**		**75**	

The iTIE as evolved since Prototype 1 to be a more robust and stable device, with the ability to be used in shallow to deeper waters for a range of regulatory applications. The current prototype is comprised of rigid, thick, secure fitting acrylic and can withstand high currents and dropping. The field deployment case and pumping case are equally strong and water resistant resembling most field monitoring equipment. The water resistance of the pumping unit still needs improving as noted below.

Although the current demonstrations were in a shallow river and from a pier, the iTIES can be used from a vessel (up to 10 m from the water surface) and in shallow or deep waters attached to a buoy or anchor in a mini-raft configuration. This latter option could be used for stormwater or effluent outfall monitoring and surface water and sediment assessments for multiple regulatory applications. The unit could also be allowed to drift in the mini-raft design as currently being done with the SEA Ring attached to a drogue in an ongoing National Oceanic and Atmospheric Administration project. These options cover the majority of freshwater and coastal water situations where Tier 2 or 3 level assessments are conducted.

From a strategic assessment approach, the iTIE should be viewed as a Tier 2 or 3 level approach for incorporation into a smart WoE study. It is a diagnostic tool to be used once Tier 1 assessments suggest chemical toxicity may be a concern. Because most sites contain a plethora of chemicals which may be contributing to toxicity, the iTIE can direct the site manager to focus on those chemicals of greatest ecological concern. In addition, the iTIE can assist in source identification of toxic chemicals whether associated with sediments, caps, ambient waters, stormwaters, or outfalls.

Conclusions and Implications for Future Research and Benefits

The proof-of-concept was established for the iTIES. This proof actually began years ago with Prototype 1, then in the past 4 yr evolved into Prototypes 2 and 3. The accomplishments of this

SEED grant would not have occurred if not for these preceding research efforts, particularly those that began with the Reible et al. 2018, SERDP project ER-2428. The iTIES prototype 3 is now a robust deployable system that allows for consistent and sensitive adjustments to pumping rates of ambient waters through the diagnostic array of resin treatments. The current battery of resins separates the following potential toxicants: ammonia, problematic heavy metals (Ag, Cd, Cu, Ni, Pb, Zn), and organics of various characteristics, including PFAS. The iTIES appears to be more sensitive at detecting ambient toxicity than the traditional laboratory-based TIE and requires fewer resources to conduct. The iTIE is a unique diagnostic tool for use in a tiered risk assessment. Its applications to a host of critical DoD concerns and SONs suggest it should become a standard diagnostic assessment technology at chemically contaminated sites.

Before the iTIES can become a standard diagnostic technology, it requires additional development. Some of these issues were discussed above, but also include the following:

- Refine the porewater sampling option – verify sediment porewater sampling zone versus surface water infiltration; gentle aeration of toxicity chamber porewater.
- Improve deployment logistics of iTIE chambers to sediments/porewater (diver vs diverless options)
- Continue to optimize resin selectivity for various target chemicals (e.g., PFOS)
- Continue assessment of deployment depth limitations (optimal would be up to depths of 20 m)
- Develop an underwater pumping container option (increases versatility for where and how long deployments can occur)
- Improve water resistance in the pumping control circuit board compartment, with a drainage valve and compartment partitioning
- Testing of early life stage fish
- Expand sublethal, chronic endpoints
- Additional field verifications (marine and freshwater)
- Environmental Security Technology Certification Program (ESTCP) verification

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Appendix A
The Evolving Steps of the iTIES

The *in situ* Toxicity Identification Evaluation (iTIE) has undergone a number of iterations to increase the robustness and reliability of the novel technology (Figure A1). Relevant aspects of the previous projects are described below.

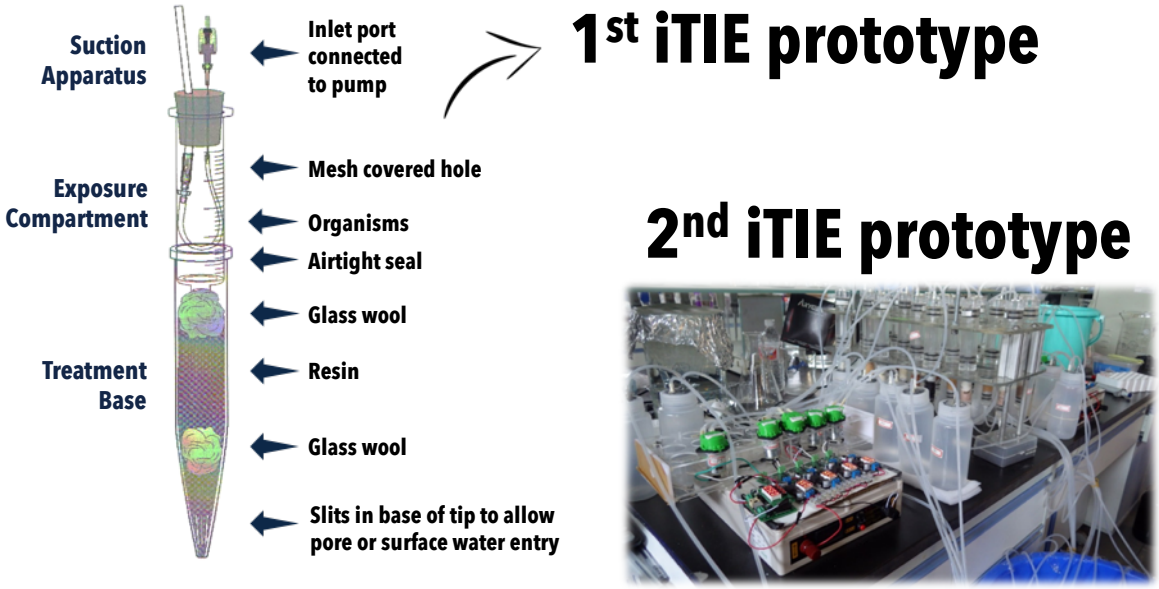

Figure A1. First and second *in situ* Toxicity Identification Evaluation (iTIE) prototypes. ***Burton and Nordstrom 2004. Environ Toxicol Chem 23:2844–2850.***

In the current project, we began with the iTIE prototype 3 (version 2017) which consists of a rectangular unit capable of housing an array of iTIE units. Each unit is equipped with an organism exposure chamber, a smaller chamber filled with a resin absorbent to fractionate porewater, surface water or effluent passing through the organism chamber, and a connection to a water collection container (Figure A2).

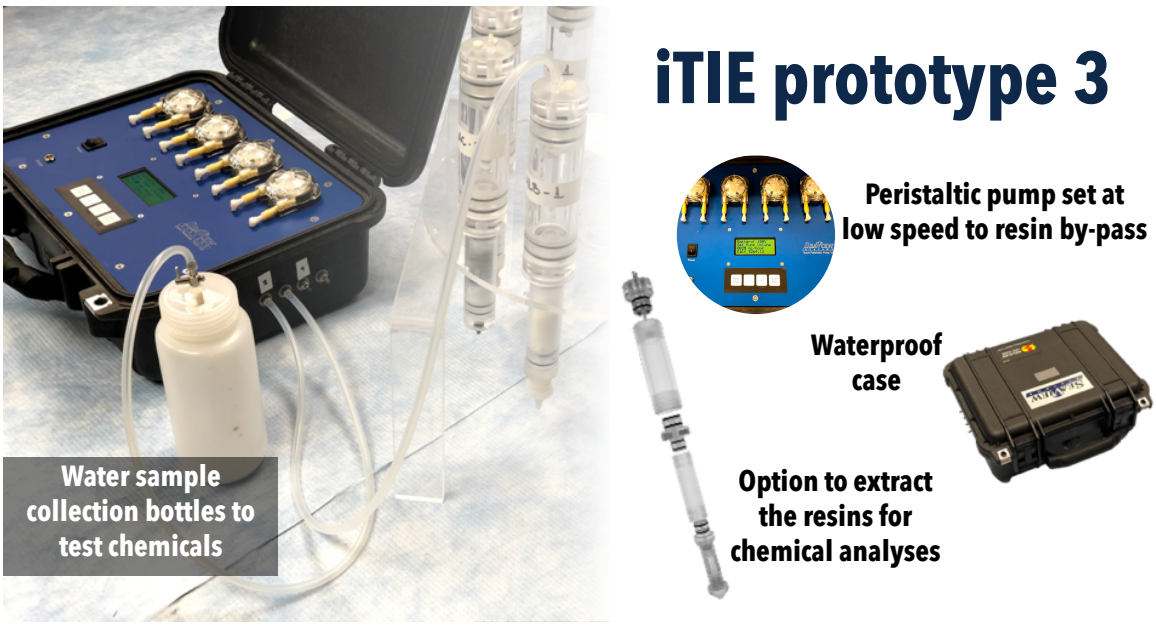

Figure A2. The *in situ* Toxicity Identification Evaluation (iTIE) prototype 3 and its specifications.

Design improvements – Steigmeyer 2015 Master's thesis

During the first 2 yr of this project, research was conducted on optimizing the design of the iTIE system (Steigmeyer 2015). Steigmeyer's research concentrated on contaminants of emerging concern, such as pharmaceuticals, antibiotics, and other personal care products as well as various metals with a goal to develop a device capable of autonomous *in-situ* TIE experiments. Two iTIE models were developed, the first experienced mechanical issues, whereas the second was successfully designed and tested in a series of laboratory fractionation tests. Tests performed demonstrated that genetic methods could be used in conjunction with the iTIE system to identify sublethal toxicity and potential to serve as early detection of molecular biomarkers (Steigmeyer 2015 and Steigmeyer et al. 2017).

Resin optimization - Meyer (2016) Master's thesis

Meyer's studies aimed to optimize the iTIE system through the evaluation of different resins (chelex, activated carbon [AC], and zeolite) and pollutant or contaminant (ammonia, Zn, Ni, and V) combinations. The experimental results indicate that at least 3 to 5 g of resin is needed for significant contaminant removal, the system flow must be maintained below 14 mL min^{-1}, and the iTIEs can transition successfully into Phase II of the TIE protocol by allowing for specific contaminant characterization.

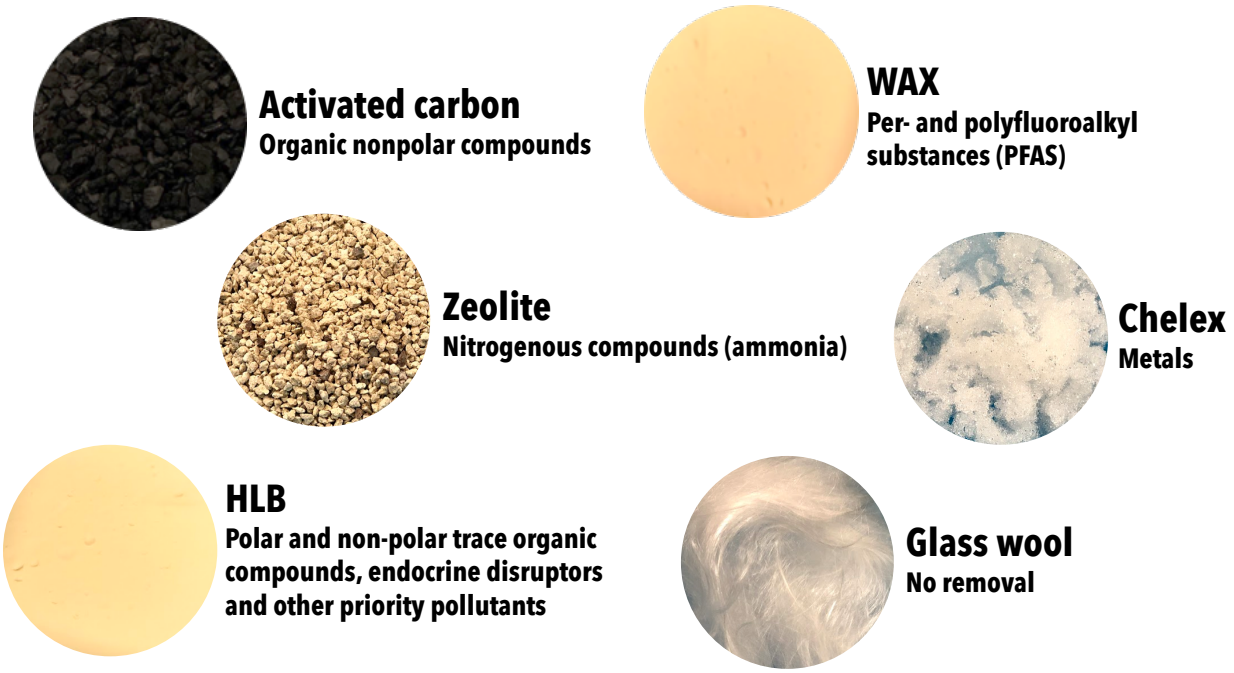

Figure A3. Different resins tested using the iTIE prototype.

Stormwater stressors – Reible et al. 2018 (ER-2428)

As a Co-Principal Investigator, G. Allen Burton, Jr. (University of Michigan) contributed to the project in 2 primary ways. First, advice was provided on stormwater sampling, including sample collection methods, quality assurance and quality control issues associated with stormwater and sediment sampling, biological effects characterizations, interpreting results, and advancing the technology of identifying which stormwater stressors are most important. This last task consisted of optimizing the iTIE method approach to determine which chemicals are the primary toxicants at test sites.

The iTIE units were acid cleaned (12% hydrochloric acid) prior to deployment. Each iTIE unit was connected to a HDPE sample bottle (1 L or 500 mL, Fisher) via silicone tubing (Cole-Parmer) with additional silicone tubing and a check valve to prevent backflow into sample bottles. The resins which extract differing types of chemicals, were preweighed (5 g each, dry) and moistened with deionized water. Resins chosen for deployment included AC (Marineland), HLB (Sigma-Aldrich), Chelex (Bio-Rad Laboratories), and glass wool (Sigma-Aldrich). AC, HLB, and Chelex were chosen as active resins whereas glass wool represents a control resin. These resins are optimal for selecting for nonpolar organics and metals – which were the primary chemicals of concern at the test site.

The iTIE systems needed to be purged of air and resins pre-rinsed. Pumps were set to 100 mL h^{-1} the night before deployment and pumping initiated. The resin HLB is viscous when wet, thus the HLB iTIE units had difficulty pumping at 100 mL h^{-1}. A high rate of pumping is initially necessary to remove any associated contaminates. Approximately 1 g of HLB was removed from each iTIE in an attempt to reduce the pumping resistance. The pump rate was increased to 200 mL h^{-1} and then 400 mL h^{-1}. This pumping rate was sufficient for all iTIE units.

The toxicity test organisms were *Americamysis bahia* and *Leptocheirus plumulosus*. These were early life stage (the most sensitive to toxicants) and obtained from Aquatic Research Organisms (Hampton, NH). The iTIEs received 10 organisms of each species the day of deployment.

Space and Naval Warfare Systems Command (SPAWAR) San Diego (CA, USA) properties served as a reference site for this study. The reference site was located off SPAWAR pier at Naval Base Point Loma whereas Paleta Creek at Cummings Road (San Diego, CA, USA) was chosen as the exposure site due to its known contamination and ease of access. Reference iTIE unit (4 glass wool resins) and exposure iTIE units (2 HLB, 2 AC, 2 Chelex, 1 glass wool) were deployed and set to pump at 100 mL h^{-1} for a 24 h exposure. iTIEs were deployed on 8 August 2017 and recovered on 9 August 2017.

Organism mortality was assessed on recovery of units. Water samples from collection bottles were taken for metals analyses (dissolved and particulate). Dissolved samples were filtered with 0.45-µm syringe filters. All metals samples were preserved to 2% trace metal grade nitric acid.

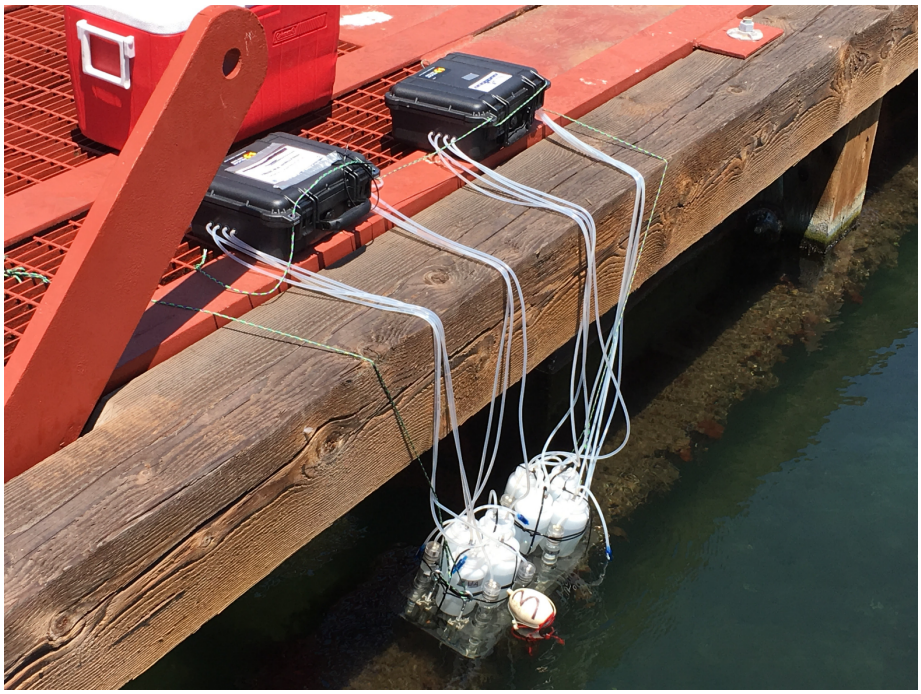

Figure A4. Field testing of the *in situ* Toxicity Identification Evaluation System at Paleta Creek in San Diego, CA, USA.

During recovery, *A. bahia* were observed eating *L. plumulosus,* which likely explains low *L. plumulosus* survival in Reference and Paleta units. Copper, the metal of most interest at Paleta, was analyzed for potential differences between resins. To accommodate data normality, a natural log transformation was performed. Results of analysis of variance indicated significance among resins ($p = 0.03$). Tukey's honest significant difference test results showed significant difference between glass wool and Chelex treatments at Paleta ($p = 0.039$). Though not statistically significant, HLB and Chelex at Paleta returned a p-value of 0.059. Pyrethroids are known to be a potential concern at this site; however, the ability to collect water samples that could be tested

with appropriate detection limits and quality assurance/quality control were doubtful, given how few laboratories can perform these analyses.

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Appendix B

Laboratory versus Field TIE Comparison. San Diego Bay, NIWC Pacific facility. February 2019

Molly Colvin, Nicholas Hayman and Gunther Rosen; Naval Information Warfare Center (NIWC) Pacific

Approach

To demonstrate the *in situ* toxicity identification evaluation (iTIE) technology, a field and laboratory effort were conducted concurrently for direct comparisons of organism performance following TIE treatments *in situ* and *ex situ*. For the field effort, 2- 50L chemtainers were deployed off Pier 169 at the NIWC Pacific facility located in San Diego, CA, USA. Each chemtainer was deployed approximately 1 ft below the surface and each held 8 iTIE units (each treatment replicated twice) and were deployed for 24 hrs (Figure 1). One chemtainer was considered an uncontaminated reference setup ("Reference"), whereas the second chemtainer had copper sheeting deployed within the chemtainer ("Copper"). Two copper sheets of approximately 3312cm^2 each were used with a copper leach rate of 162 µg/cm^2 copper per day (Earl et al. *in prep*) for an approximate loading of 10,797 µg/L/d.

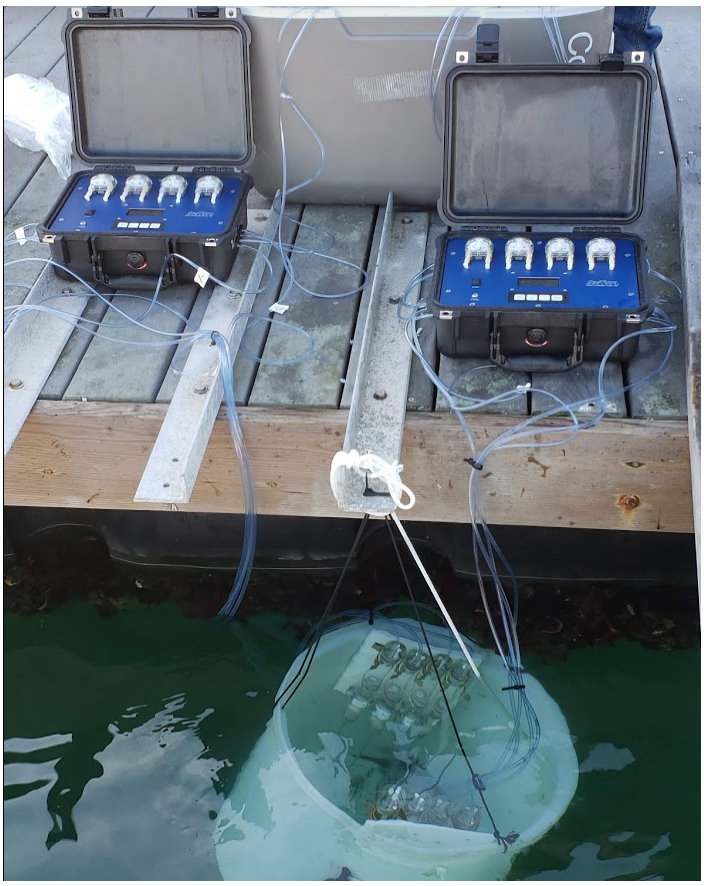

Figure B1. Deployment setup for the Reference Chemtainer with the iTIE units.

Organisms were placed into the iTIE chambers and deployed on 19 February 2019. Organisms utilized and their respective test acceptability criteria, are listed below:

- 24-h acute survival toxicity test using the marine rotifer, *Brachionus plicatilis* (L strain; ASTM International 1998)
    - ≥ 90% survival in controls
- 48-h chronic larval development test using the Mediterranean mussel, *Mytilus galloprovincialis* (US Environmental Protection Agency 1995)
    - ≥ 90% normal shell development in controls
- 96-h chronic embryo-larval development test using the purple sea urchin (*Strongylocentrotus purpuratus*, US Environmental Protection Agency 1995)
    - ≥ 80% normal development in controls

Equipment and organisms were recovered from Pier 169 on 20 February 2019 and contents of the organism exposure chamber were filtered through a 25 µm Nitex screen. Rotifers were enumerated on recovery, whereas the mussel and urchin larvae were transferred into 2 scintillation vials using water collected from the iTIE organism exposure chamber. These vials were placed into a 15 °C incubator for the duration of the tests. One vial from each treatment was terminated at 48 hrs (for mussel larval development evaluation) and the second terminated at 96 h (for urchin larval development evaluation). Termination of an exposure included the addition of 10% buffered formalin and subsequent microscopic evaluation for normal development.

In addition, water samples that were collected from the iTIE units were sent to Weck Labs, Inc. for analytical chemistry. Four treatments were evaluated for the iTIE units in the field:

- Glass wool (control);
- HLB;
- C18;
- Chelex Resin.

For the laboratory-based TIE evaluation, water grab samples were collected from within the field deployed chemtainers at approximately 18 h following deployment. Exposures were initiated in 20 February 2019 with the following TIE treatments:

- Base (no manipulation);
- 0.45 µm filtration;
- Aeration;
- C18 SPE column;
- Ethylenediaminetetraacetic acid (EDTA) addition.

Results

For the field-based efforts, no rotifers were recovered from the exposure chambers in either the Reference or Copper chemtainers. Mussel larvae recovery was low in the Reference replicates (mean ± standard deviation was 29 ± 23 larvae/vial). Urchin recovery was much higher in general, with all but 2 vials resulting in the requisite 100 counted larvae prescribed by the standard method. For the mussel embryo-larval development test, the control treatment (Glass wool) associated with the reference chemtainer failed to meet the ≥ 90% normal shell development in surviving controls, with the normal development only reaching 67.5%. However, this treatment showed a higher percentage than normal than all other treatments performed (Figure B2, Table B1). The copper chemtainer exposures only had mussel larvae recovered from the Chelex treatment; which showed similar normal development compared to the reference chemtainer indicating the removal of copper in solution prior to the organism exposure chamber. In addition, 1 of the replicates from the HLB treatment had the material leak through into the exposure chamber, rendering this replicate un-usable.

For the urchin embryo-larval development exposures, the control treatment (Glass wool) associated with the reference chemtainer met the ≥ 80% normal shell development in surviving controls, with normal development of 85.7% (Figure B3, Table B1). Other treatments in the reference chemtainer performed below test acceptability criteria. Comparatively, the copper exposed iTIE units showed complete abnormal development save for a few normal larvae in the Chelex treatment. It was observed that many of the eggs that were introduced to the exposure chambers did not progress much beyond the first few stages of cellular division following fertilization suggesting high concentrations of copper. For treatment specifics, for all endpoints, a possible toxic effect of the C18 treatment was observed, as normal development was reduced in the reference exposure as well. This toxic effect was not observed in the laboratory exposure (see below, Table B2), where the C18 treatment in the Reference water resulted in high normal development for all species evaluated.

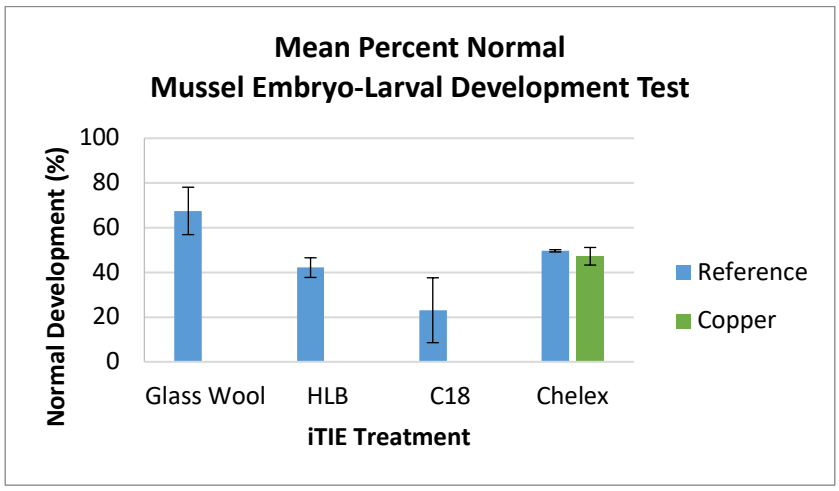

Figure B2. Mean percent normal for the mussel embryo-larval development test for the reference and copper chemtainer exposures *in situ*.

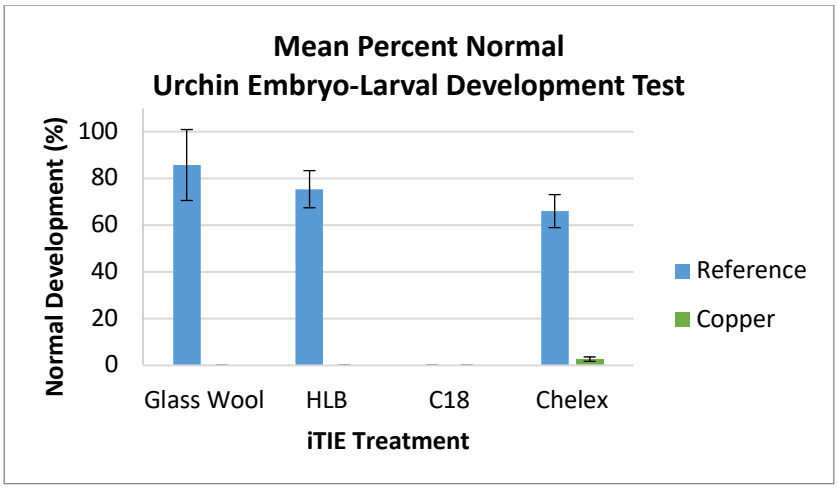

Figure B3. Mean percent normal for the urchin embryo-larval development test for the reference and copper chemtainer exposures *in situ*.

Table B1. Summary of *In Situ* Results – Mussel and Urchin Embryo-Larval Development.

Chemtainer Exposure ID	iTIE Treatment ID	Mussel % Normal Dev (48 hrs)		Urchin % Normal Dev (96 hrs)	
		Average	StDev	Average	StDev
Reference	Glass Wool	67.5	10.6	85.7	15.2
	HLB	42.1	4.4	75.4	7.9
	C18	23.1	14.5	0.0	0.0
	Chelex	49.6	0.5	66.0	7.1
Copper	Glass Wool	0.0	0.0	0.0	0.0
	HLB*	0.0	--	0.0	--
	C18	0.0	0.0	0.0	0.0
	Chelex	47.2	3.9	2.7	0.9

*Only 1 replicate recovered.
DEV = development; St Dev = standard deviation.

For the laboratory-based efforts, the rotifers in the reference water met test acceptability criteria of 90% survival in all TIE treatments except for the EDTA addition, which had 85% survival (Figure B4, Table B2). For the copper water exposures, the EDTA and C18 column reduced toxicity relative to the unmanipulated "Base" sample. Aeration and 0.45 µm filtration of the sample only slightly reduced some toxicity relative to the base sample.

For the mussel embryo-larval development test, all treatments from the reference water sample met the test acceptability criteria of ≥ 90% normal shell development in surviving controls (Figure B5, Table B2). Toxicity identification evaluation treatments performed on the copper water samples showed no normal surviving organisms except for the EDTA treatment, with 97%

normal development. Ethylenediaminetetraacetic acid binds with metals and renders the metal non-bioavailable to the organisms, thus reducing toxicity.

For the urchin embryo-larval development test, all treatments from the reference water sample met the test acceptability criteria of ≥ 80% normal shell development in surviving controls (Figure B6, Table B2). Similar to the mussel test, TIE treatments performed on the copper water samples showed no normal surviving organisms except for the EDTA treatment, with 88.5% normal development. With regard to specific treatments, the C18 treatment reduced toxicity for the acute rotifer exposure, but did not for both chronic larval-development endpoints. The median lethal concentration (LC_{50}) for rotifers (80 µg/L [Snell and Persoone 1989; ASTM International 1998; Arnold et al. 2010]), is higher than the median effect concentration (EC_{50}) for mussels and urchin (8.0 and 14.9 µg/L, respectively; Rosen et al. 2008). This suggests that C18 was able to provide some protective effects from copper toxicity, but not as much as EDTA.

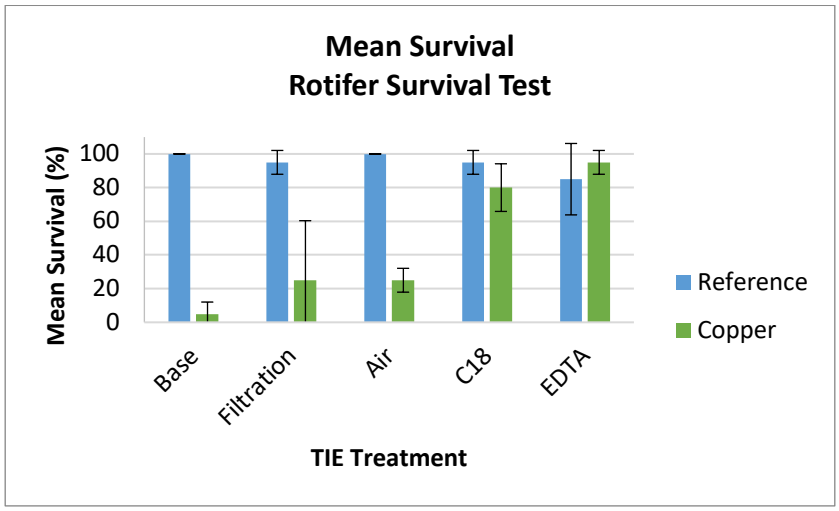

Figure B4. Mean percent survival for the urchin embryo-larval development test for the reference and copper chemtainer water exposures *ex situ*.

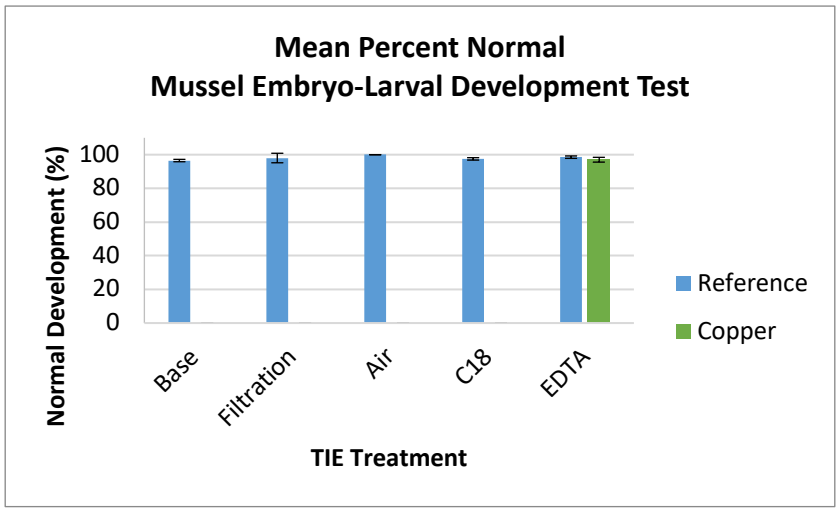

Figure B5. Mean percent normal for the mussel embryo-larval development test for the reference and copper water exposures *ex situ*.

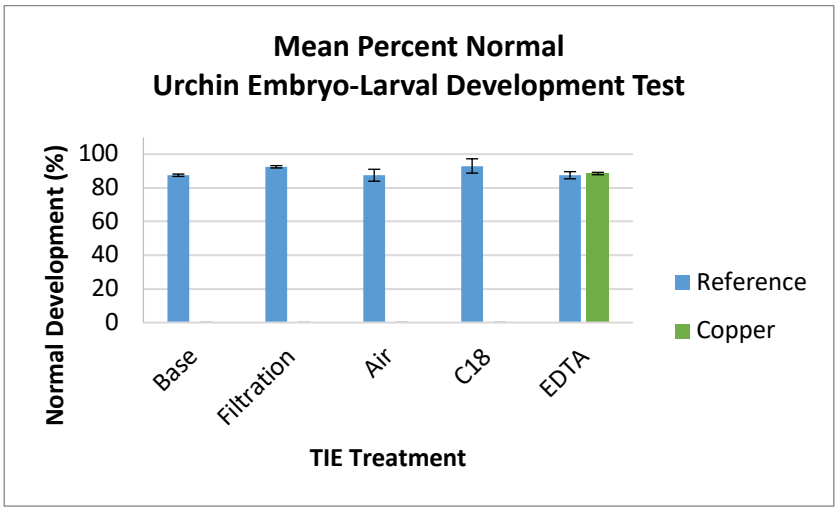

Figure B6. Mean percent normal for the urchin embryo-larval development test for the reference and copper chemtainer water exposures *ex situ*.

Table B2. Summary of *Ex Situ* Results – Rotifer, Mussel and Urchin Embryo-Larval Development.

Exposure ID	TIE Treatment ID	Rotifer % Survival (24 hrs)		Mussel % Normal Dev (48 hrs)		Urchin % Normal Dev (96 hrs)	
		Average	StDev	Average	StDev	Average	StDev
Reference	Base	100.0	0.0	96.5	0.7	87.5	0.7
	Filtration	95.0	7.1	98.0	2.8	92.5	0.7
	Air	100.0	0.0	100.0	0.0	87.5	3.5
	C18	95.0	7.1	97.5	0.7	93.0	4.2
	EDTA	85.0	21.2	98.5	0.7	87.5	2.1
Copper	Base	5.0	7.1	0.0	0.0	0.0	0.0
	Filtration	25.0	35.4	0.0	0.0	0.0	0.0
	Air	25.0	7.1	0.0	0.0	0.0	0.0
	C18	80.0	14.1	0.0	0.0	0.0	0.0
	EDTA	95.0	7.1	97.0	1.4	88.5	0.7

Dev = development; StDev = standard deviation.

Summary

Mussel recovery from the *in situ* exposure was too low to be reliable for the evaluation of iTIE methodologies. Urchin recovery did appear to be adequate, although development was not as "clean" (meaning the pluteus larvae were not as well developed) in the iTIE setup compared to the laboratory exposure. This may have been due to the manipulations and physical stress that were placed on the developing larvae when transferred from the iTIE exposure chambers into

scintillation vials after 24 h of deployment. These issues have been noted for the both species in previous *in situ* exposures (such as in the SEA Ring).

Although the rotifer has a 24 h test duration, which is compatible with the current duration of deployment of the iTIE units, an issue that arose was the difficulty associated with hatching the marine rotifer and the lack of being able to obtain sufficient numbers of rotifers within the appropriate time for test initiation. The use of a more robust organism, such as the mysid shrimp, *Americamysis bahia*, may be preferable to the use of the rotifer, or either larvae species for that matter, as mysids have an alternate 24 h acute endpoint as well, and have shown higher recovery in previous iTIE and other *in situ* deployments.

Original leachate estimates of the copper plates may have resulted in an overabundance of copper present than the iTIE setup was able to handle (primarily demonstrated through the breakthrough of the Chelex treatment). The Chelex resin in the iTIE treatment chambers were green, suggesting that it had bound copper and possibly become saturated, resulting in copper getting through to the exposure chamber and resulting in a toxic exposure to the organisms. The dilution/mixing of the copper-leach material and overlying water was not well confirmed and therefore, future deployments using the copper sheets should consider using fewer copper sheets and a possibly a more robust species, such as mysid shrimp.

A potential downside to using the mysid shrimp is the inability to co-deploy them with other organisms due to the difference in test duration or the potential for predation of the other species resulting in poor interpretation of results. This current deployment showed a successful deployment of urchins and mussel larvae in the same chamber; however, in practice, this was complicated due to the difference in test duration (i.e. 48 and 96 h test duration for mussels and urchins, respectively).

An additional consideration is to ensure that treatments being performed in the iTIE are complementive to concurrent TIE sample manipulations being performed on the samples. An example of a good comparison was the C18 treatment which was performed on both *in situ* and *ex situ* exposures/samples. It is difficult to compare the Chelex resin which was evaluated through the *in situ* exposures relative to the EDTA treatment performed in the laboratory.

References

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Snell TW, Persoone G. 1989. Acute toxicity bioassays using rotifers. II. A freshwater test with *Brachionus rubens. Aquat Toxicol* 14:81–91.

US Environmental Protection Agency. 1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to West Coast marine and estuarine organisms. EPA/600/R-95/136. Environmental Monitoring and Support Laboratory, Cincinnati, OH.

Appendix C

Per- and Polyfluoroalkyl Substances (PFAS)

Laboratory screening test

The objective of this initial screening assessment was to evaluate the effectiveness of the *in situ* toxicity identification evaluation (iTIE) technology in identifying toxicity of per- and polyfluoroalkyl substances (PFAS). The iTIE deployment was conducted within a chemtainer (50 L) containing 100 ng/L of both perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) diluted in deionized water. Native PFOS and PFOA analytes were obtained from Sigma-Aldrich. Stock standard solutions (100 ng/L) of native and labelled PFASs were prepared in 96:4% (v/v) methanol:water.

The iTIE units were acid cleaned (12% hydrochloric acid) prior to deployment. Each iTIE unit was connected to a HDPE sample bottle (1 L, Fisher) via silicone tubing (Cole-Parmer) with additional silicone tubing and a check valve to prevent backflow into sample bottles. The resins which extract differing types of chemicals, were preweighed (3 g each, dry) and moistened with deionized water or methanol. Resins chosen for deployment included activated carbon (AC) (Marineland), Oasis HLB (Waters), Oasis WAX (Waters), and glass wool (Sigma-Aldrich). Activated carbon, HLB, and WAX were chosen as active resins whereas glass wool represents a control resin. These resins are optimal for selecting organic acids.

The iTIE units were deployed and set to pump at 25 mL/h for a 24 h exposure. Ten organisms of *Daphnia magna* were placed inside each exposure chamber to assess the acute effects of water processed by each iTIE. Perfluorooctanesulfonic acid and PFOA levels collected in the exposure bottles were analyzed by a high-performance liquid chromatography tandem mass spectrometry system (HPLC-MS/MS) at Dr. Chris Higgins' laboratory (Colorado School of Mines, USA). Laboratory results show that all resins removed >93% of PFOA and PFOS compared to the glass wool control. *Daphnia magna* survival was not affected by PFAS at concentrations of 100 ng/L. However, both replicates from HLB treatment had the material leak through into the exposure chamber, possibly causing toxicity of *D. magna* (survival of 50%). Survival of *D. magna* for Oasis WAX was 95%, whereas 85% of the organisms survived using AC.

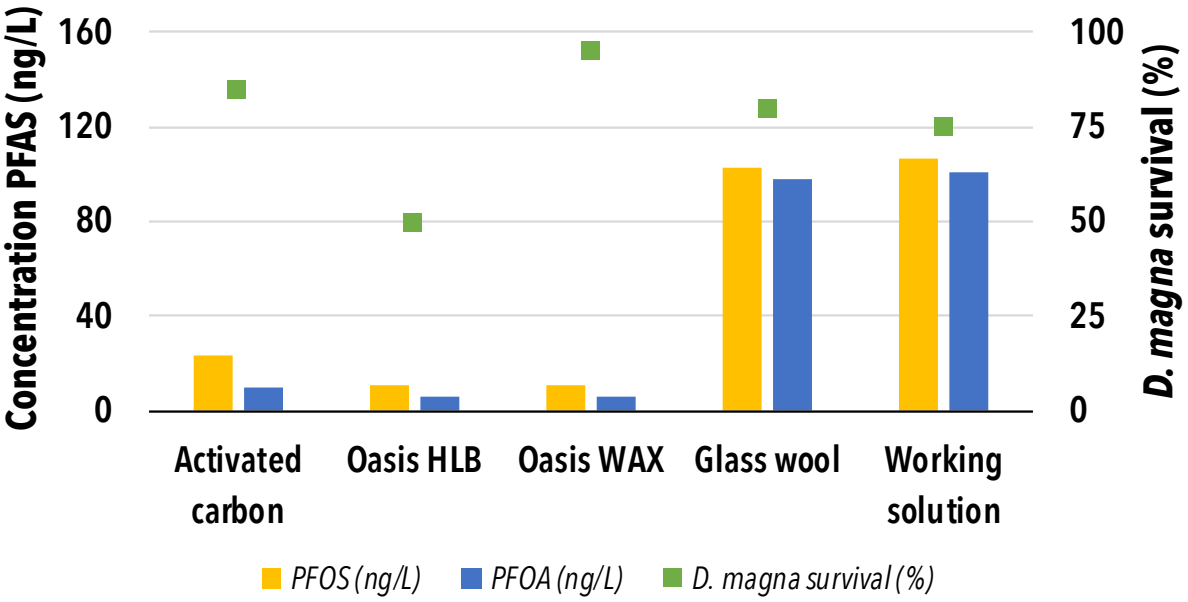


Figure C1. Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) mean concentrations after extraction using different commercially available resins and its acute effects in *Daphnia magna*.

*Only 1 replicate of the Oasis HLB recovered.

Appendix D

Additional Photographic Documentation

Figure D1. Large organism chamber for fish testing.

Figure D2. Deployment case setup in the Clinton River, MI, USA for PFAS toxicity identification evaluation.

Figure D3. Pump unit, collection bottles, and iTIE units setup inside the deployment case at the Clinton River, MI, USA.

Appendix E
iTIE Pump Unit and iTIE Deployment Case Development Status

This document provides a high-level summary and documentation of components of SeaView Systems' contributions to the Department of the Army contract W912HQ18C0019, "Proof-of-Concept for the *in situ* Toxicity Identification Evaluation (iTIE) Technology for Assessing Contaminated Sediments, Remediation Success, Recontamination and Source Identification." SeaView Systems designed and assembled pumping units (iTIE pump units) for sample collection and field transport cases (iTIE deployment case) to create an all-in-one portable laboratory for this contract.
The iTIE pump units are comprised of 4 synchronous peristaltic pumps that are housed in a Pelican 1400 Protector case along with control electronics. The pumps are controlled by a microprocessor which allows for precise control of the pumping rate and stores the calibration. An integrated digital display shows the approximate volume of water pumped by 1 unit. The custom circuits as designed for the iTIE pump units are shown in the diagrams in Appendix A of this document. These are:

- iTIE control board with integrated user-interface display
- iTIE motor driver

Each iTIE deployment case is housed in a Pelican iM2750 storm travel case and includes space for the iTIE pump unit and additional components as required for stationing the iTIE deployment case for sample collection. These include:

- Space for a single iTIE pump unit (iTIE pump unit can also be removed)
- Space for up to 100 m of tubing under the pump unit
- Legs and footpads with necessary hardware to attach to case for water deployment which are stowed in the iTIE deployment case for transport
- Holders for 4 collection bottles and 4 iTIE water column spikes
- Tubing and barbed connectors for attaching the iTIE water column spikes

After initial field trials of a prototype unit comprised of the combination of an iTIE pump unit and iTIE deployment unit, conducted to identify areas where improvements could be made, modifications were made to 4 aspects:

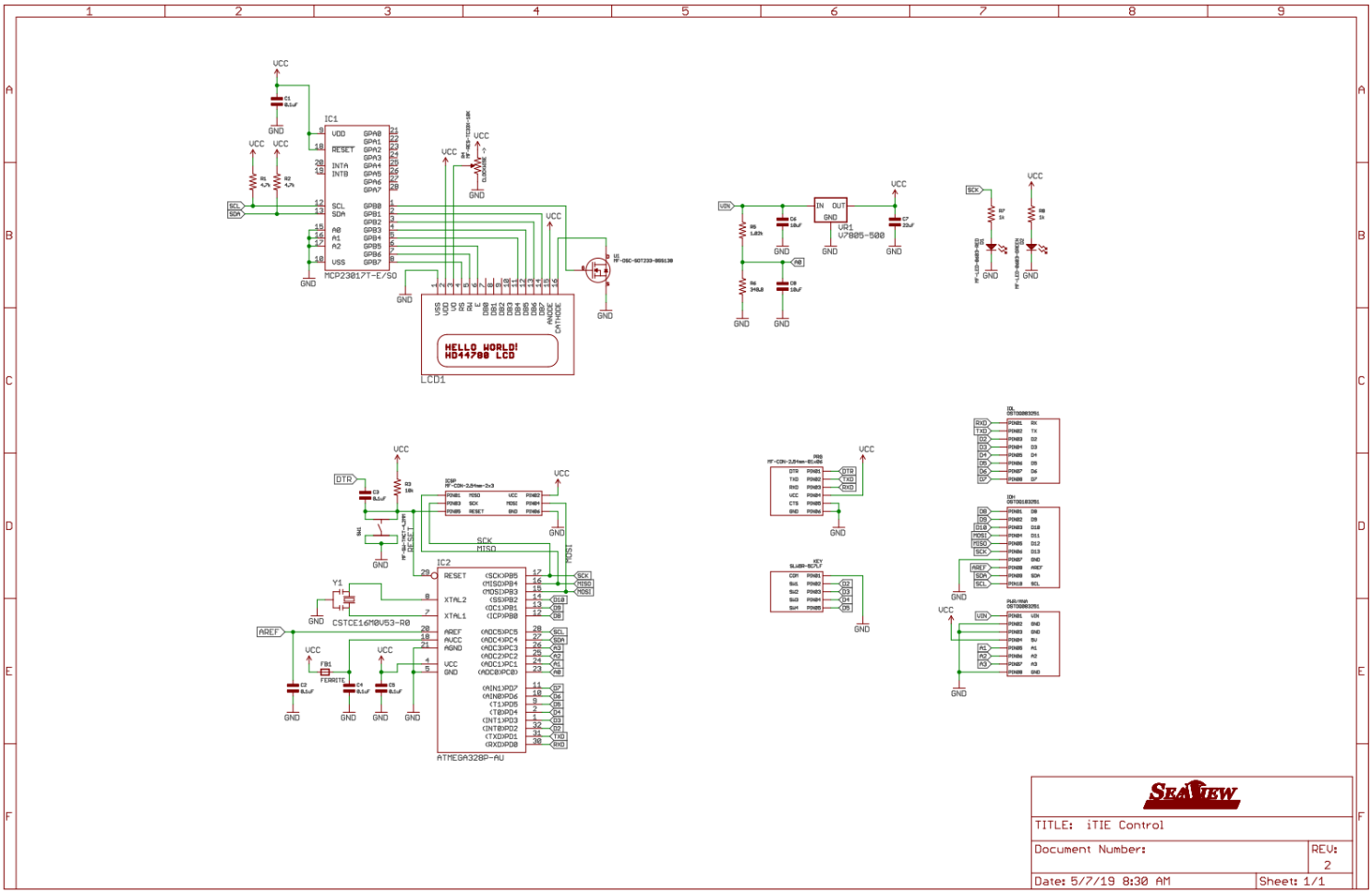
- The pump's circuit boards were conformally coated to reduce the likelihood of condensation or water intrusion damage to the control electronics
- An extra tie-down was added to secure the legs more when stored or in transport
- The holder for the pump's battery was fortified
- Metal clips were replaced with plastic for holding the iTIE spikes to avoid corrosion

iTIE Pump Unit and iTIE Deployment Case Possible Future Enhancements

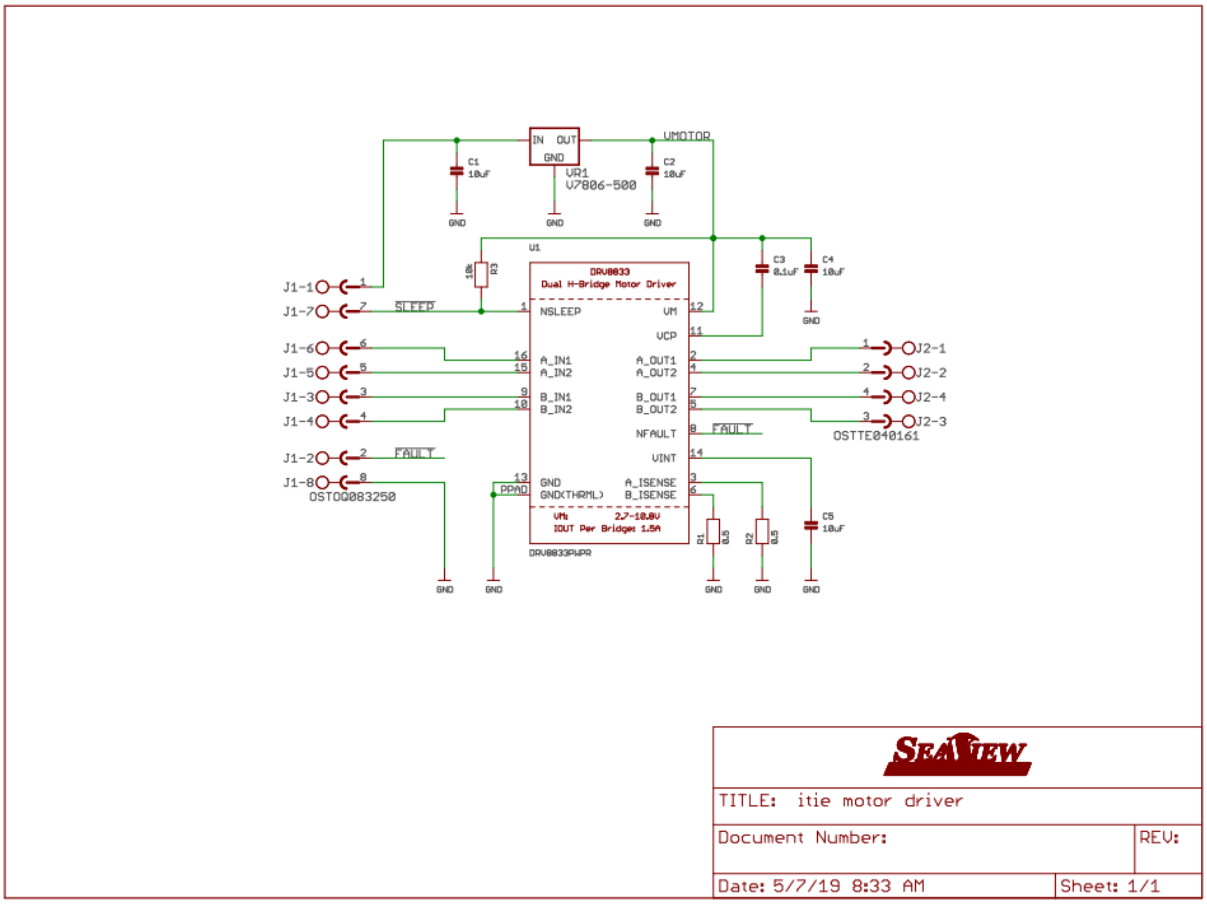
It is suggested that any future work should consider the inclusion of the following improvements to the systems:

- Development of a Field User Manual that encompasses guidelines for field deployment, transport, diagnosing possible error conditions, and calibration procedures among other topics
- Improvements to case design and internal electronics water resistance
- Improvements to case design to reduce weight and improve ease of transport
- Deployment case design improvements to allow more pumping units in a single case or modular system to allow individual pumps rather than ganged pumps
- Design change to facilitate calibration sequences of individual pumps independently, control peristaltic pump rates independently of one another and provide greater pump rate control, start and stop timing control, serial pump activation sequences, etc. as required
- Addition of monitoring and alert system (perhaps using ultrasonic sensor or load cell to detect bottle weight) to provide feedback in the event of pump issues such as reduced or mismatched flow, pump failure or other error conditions
- Addition of wireless control to turn on/off pumps and provide alerts (via cellular, WIFI, or Bluetooth connection) such as flow rate monitor or error condition alerts as implemented above
- Development of general-purpose monitoring graphical user interface showing pump status, leak detection, etc. in graphical display
- Smartphone App to support above control, monitoring, and alerts system
- Other enhancements TBD

Custom Circuit Board Design Documents



iTIE control board with integrated user-interface display



iTIE motor driver board