EXECUTIVE SUMMARY

In Situ Toxicity Identification Evaluation (iTIE) Technology for Assessing Contaminated Sediments, Remediation Success, Recontamination and Source Identification – Phase II

G. Allen Burton Jr.
Austin Crane
Haixiang Mao
Seth Strauss
Elizabeth Nichols
Aiden Nicholson
Megan Roe
Grace Thompson
University of Michigan

Bart Chadwick
Coastal Monitoring Associates

Gunther Rosen
Marienne Colvin
Naval Information Warfare Center Pacific

Edward Verhamme
Jennifer Daley
LimnoTech, Inc.

Timothy Crandle
SeaView Systems, Inc.

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SERDP Project ER18-1181

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Project: ER18-1181

TABLE OF CONTENTS

Page

ACRONYMS AND ABBREVIATIONS

AChE	acetylcholinesterase
AFDW	ash-free dry weight
CoC	Chemical of concern
DO	dissolved oxygen
EPA	Environmental Protection Agency
GAC	granular activated carbon
iTIE	*in situ* Toxicity Identification Evaluation
iTIES	*in situ* Toxicity Identification Evaluation System
PAH	polycyclic aromatic hydrocarbon
PFAS	per- and polyfluoroalkyl substances
SEED	SERDP Exploratory Development
SERDP	Strategic Environmental Research and Development Program
SON	Statement of Need
TIE	toxicity identification evaluation

ACKNOWLEDGEMENT

The *in situ* Toxicity Identification Evaluation (iTIE) concept and first prototype iteration were originally developed by Burton and Nordstrom (2004). Subsequent prototype and protocol refinements were made in thesis studies completed by Steigmeyer (2015), Meyer (2016), and Nichols (2023), as well as a practicum completed by Calloway (2022). Phase I of this project was funded by the Strategic Environmental Research and Development Program (SERDP; Contract No. W912HQ18C0019, Project No. ER18-C4-1181), and was completed in 2019. The following report details Phase II of the project, also supported by SERDP (Contract No. Contract No. 21-C-0052, Project No. ER18-1181). This research would not be possible without the support of personnel from the Burton Lab, Naval Information Warfare Center Pacific, Coastal Monitoring Associates, and other University of Michigan School for Environment and Sustainability research labs and administrative departments. Dr. Karen Alofs of the University of Michigan provided facilities, equipment, and advice for fish care. Dr. Andrew Gronewold of the University of Michigan provided advice for data analysis. Sally Petrella of Friends of the Rouge provided advice for field deployments.

1.0 INTRODUCTION

This report details the second phase of SERDP Exploratory Development (SEED) project ER18-1181. SERDP has previously issued Statements of Need (SON), calling for "innovative approaches for both monitoring and implementing *in situ* remediation of impacted aquatic sediments" that "ultimately reduce costs." At sites with multiple stressors present, there is a need for realistic, cost-effective *in situ* tools that can quantitatively demonstrate causal linkages between chemical classes and adverse biological effects. At present, stressor-toxicity linkages are commonly determined through qualitative methodologies. Existing quantitative methods, like the U.S. Environmental Protection Agency (EPA) toxicity identification evaluation (TIE) method, are often confounded by excessive sample manipulation and fail to capture realistic site conditions. These challenges hinder effective management and remediation decision-making, which risks the unaddressed impairment of critical ecosystems and the unnecessary loss of time and public resources.

The overall purpose of this project was to develop and refine a novel *in situ* monitoring technology that could be used to establish stressor-causality linkages via realistic, cost-effective exposures. The *in situ* Toxicity Identification Evaluation System (iTIES) is a biological fractionation technology in which site water is continuously sampled, oxygenated, fractionated by an array of resins, and exposed to test organisms *in situ*. Different resins are selected for each iTIES deployment depending on site knowledge (i.e., chemicals expected to be present). This iTIES provides direct quantitative linkages between the presence of chemicals and observed toxicity. After Tier 1 of an ecological risk assessment, in which chemical toxicity is detected as site stressor, the iTIES can be utilized in a Tier 2 or 3 assessment to determine which chemical of concern (CoC) classes are predominantly causing toxicity and their relative toxicity contributions.

The following tasks were completed in Phase I of this project:

1) The development of the Prototype 3 iTIE Unit, an acrylic two-chamber unit that is robust, easily deployable and broadly applicable.
2) Proof-of-concept deployments of the iTIE technology in several lab and field-based applications.
3) Resin optimization studies, where several candidate resins, ideal resin volumes, and optimal flow rates were identified and tested.
4) A cost comparison investigation, where it was proven that iTIE methodologies require approximately half the time to complete compared to traditional U.S. EPA TIE methods.

2.0 OBJECTIVES

The overall objective of Phase II, the current project iteration, was the continued development and verification of the iTIES as a widely applicable technology and testing protocol for incorporation into weight-of-evidence site characterization studies. At the end of Phase I, the following sub-tasks were identified as key focus areas for Phase II:

Task 1: refinement of the iTIE prototype to allow for porewater sampling and diverless deployment.

Task 2: the testing of early life stage fish.

Task 3: an expansion of available sublethal chronic endpoints in invertebrate test organisms.

Task 4: a continuation of resin optimization efforts.

Task 5: additional field verifications in marine and freshwater environments.

Task 6: development of a decision-making framework to guide iTIES implementation.

3.0 TECHNICAL APPROACH

TASK 1: REFIEMENT OF THE ITIE PROTOTYPE TO ALLOW FOR POREWATER SAMPLING AND DIVERLESS DEPLOYMENT

This task built upon the Prototype 3 iTIE Unit from Phase I of the SEED project. A full iTIE system was developed using the Prototype 3 iTIE Unit as its foundation. The latest iTIES prototype was designed to be suitable in a wide variety of deployment settings and study goals. The ability to sample and test porewater was a key focus of Phase II.

Site porewater was continuously collected using a porewater sampler known as the Trident probe (Chadwick et al., 2003). The Trident probe could be installed and retrieved diverlessly in waters up to 10 meters deep. The Trident probe's ability to collect porewater while limiting surface water infiltration was a focus of study during this SEED project. Porewater often contained CoCs at far greater concentrations than found in surface water. Additionally, chemical stressors in surficial sediments disproportionately impacted benthic macrofaunal health and community biodiversity, compared to other ecological compartments (Brown et al., 2000; Reynoldson, 1987). The inadvertent drawdown of surface water could dilute CoC concentrations in sampled porewater, which may have confounded conclusions regarding the stress caused by sediments. To address this, the research team conducted an in-lab tracer dye study, where the Trident was assessed at a variety of sampling depths in sediments of varying textures.

Given the low dissolved oxygen (DO) content of porewater, another priority for Phase II was the construction of a system capable of providing gentle aeration to sampled water. The latest iTIES prototype included an oxygenation system, through which water flowed after it was sampled by the Trident probe. After porewater was aerated, it was exposed to resins and test organisms contained in Prototype 3 iTIE units, and collected in sample bottles for post-exposure chemistry analysis. Water movement through the iTIES was driven by a series of peristaltic pumps, which could be precisely programmed to ensure optimal oxygenation and resin exposure time. Several tubing materials were assessed for efficacy for the oxygenation system, specifically when aerating water containing dissolved sulfide.

All iTIES components were housed within easily transportable Pelican coolers. The iTIES prototype underwent iterative assessment and optimization through a series of laboratory tests. Following optimization, the prototype was used in a series of field deployments at a variety of sites, both marine and freshwater.

TASK 2: TESTING OF EARLY LIFE STAGE FISH

Early life stage fish were an important class of organisms for toxicity testing due to their sensitivity to chemicals and significance for ecosystem structure. The objective of Task 2 was to validate embryo-larval fish as viable test organisms for iTIE applications and to confirm teratogenicity as a detectable endpoint in brief exposure periods. This was primarily accomplished through field deployments utilizing embryo-stage fish as test organisms, with survival, teratogenicity, and ash-free dry weight (AFDW) as key endpoints. A series of lab-based toxicity tests were also conducted using embryo-stage fish prior to the use of fish in field deployments.

TASK 3: EXPANSION OF AVAILABLE SUBLETHAL CHRONIC ENDPOINTS IN INVERTEBRATE TEST ORGANISMS

In Phase I of this SEED project, proportion of survival was used as the primary toxicity endpoint. Task 3 necessitated the addition of available sublethal chronic endpoints for usage in an iTIES deployment. Section 5.2 of the full report describes efforts to use acetylcholinesterase (AChE) activity in freshwater amphipods (*Hyalella azteca*) as an endpoint to detect toxicity due to exposure to organophosphate pesticides. *H. azteca* adults were exposed to water spiked with chlorpyrifos in a series of in-lab iTIE experiments using various resins targeting organics, including C18 and Oasis HLB. Resulting AChE specific activity was quantified in bioassays.

In addition, several chronic endpoints were assessed for application in various lab- and field-based iTIE deployments. Quantifiable chronic endpoints that were evaluated included reproduction timing, reproduction rates, and growth as AFDW.

TASK 4: CONTINUATION OF RESIN OPTIMIZATION EFFORTS

The iTIES was intended to be applicable at a broad range of sites impacted by varying chemical stressor combinations. Resins needed to be able to selectively target classes of CoCs while themselves not inducing stress to organisms. Task 4 entailed the continued testing of diagnostic resins for iTIE usage. A standardized *Daphnia magna* sub-chronic toxicity test method was used to assess potential toxicity caused by each resin. Section 4.2 of the full report details efforts to explore new resins for adsorption of per- and polyfluoroalkyl substances (PFAS). Section 4.3 of the full report describes an investigation of conditioning methods for Chelex, a resin effective in binding heavy and transitional metals, but also noted for causing stress in freshwater organisms. Section 4.4 of the full report explores various conditioning methods for granular activated carbon (GAC), a general adsorbent for heavy metals, organics of mixed polarities, and natural stressors like sulfides, though GAC has also been found to negatively impact organisms if unproperly conditioned.

TASK 5: ADDITIONAL FIELD VERIFICATIONS IN MARINE AND FRESHWATER ENVIRONMENTS

Task 5 necessitated the completion of additional field deployments of the iTIES at a variety of field sites. Sites were chosen and deployments were designed based on different areas of focus for iTIES optimization and verification. In chronological order of deployment:

1) The iTIES was field-tested at several relatively clean freshwater sites to verify the performance of technological components, including Third Sister Lake and Fleming Creek in Ann Arbor, MI.
2) Sexton and Kilfoil Drain in Taylor, MI, receives effluent from the Detroit Metropolitan Airport during high flows. CoCs associated with airports, including ammonia and PFAS, were expected to be present in sediment in the drainage. An iTIE exposure was completed at the drainage using *Pimephales promelas* embryos.
3) Paleta Creek in National City, CA, is a marine/estuarine site with previous detections of heavy metals, pesticides, polycyclic aromatic hydrocarbons, and dissolved sulfide. Site characteristics including depth, hydrology, and chemical mixtures made Paleta Creek ideal for iTIES prototype testing. In the most recent iTIES deployment, porewater was sampled, fractionated, and exposed to *Atherinops affinis* embryos and *Americamysis bahia* larvae for 8 hours. Following the observation of significant toxicity at the site, fractionated water samples were transported back to the lab, vigorously aerated, and exposed to *A. affinis* embryos for 48 hours for additional insight. Sulfide concentrations were quantified in porewater before and after exposure to the iTIES oxygenation coil.
4) Clark's Marsh in Oscoda, MI, is a freshwater site with a legacy of PFAS impacts. The site was chosen to verify the ability of the iTIES to detect PFAS toxicity. Due to logistical constraints, an *ex situ* deployment of the iTIES was completed utilizing *P. promelas* embryos and *Chironomus dilutus* larvae as test organisms.
5) The Rouge River in Detroit, MI, is a freshwater stream with a highly industrialized and urbanized watershed. Multiple chemical stressors including metals and organic toxicants have historically been detected in the sediment near the river's mouth. An iTIES deployment was completed near the river's mouth using *P. promelas* embryos and *C. dilutus* larvae.

TASK 6: DEVELOPMENT OF A DECISION-MAKING FRAMEWORK TO GUIDE ITIES IMPLEMENTATION

Specific conditions needed to be satisfied to successfully integrate the iTIES into a successful higher-tiered site assessment. Task 6 required the development of a decision-making framework to help site managers determine if and how to incorporate the iTIES into an effective assessment.

4.0 RESULTS AND DISCUSSION

TASK 1: REFINEMENT OF THE ITIE PROTOTYPE TO ALLOW FOR POREWATER SAMPLING AND DIVERLESS DEPLOYMENT

The current iTIES prototype is a robust, broadly usable field technology that allows for porewater sampling and testing, gentle aeration of sampled water, diverless deployments at depth, and applicability in a wide range of environments. Through a series of lab tests, field deployments, and iterative refinements, the current system prototype has been evaluated and successfully deployed in a variety of settings.

The Trident porewater sampler was found to effectively sample porewater without significant surface water infiltration in multiple sediments. A minimum sampling depth of three inches was needed to prevent infiltration and preserve porewater sample integrity. However, the Trident was unable to sample porewater from one sediment with a high proportion of finer particles. Further investigation is recommended to determine whether the dynamics observed with this sediment are likely to occur with other clayey sediments.

The iTIES oxygenation system was capable of oxygenating anoxic porewater, even when initially saturated with dissolved sulfide. Based on findings from in-lab experiments, the oxygenation system was built using gas-permeable silicone to efficiently deliver oxygen to porewater and oxidize dissolved sulfides into less toxic sulfur forms.

Additional enhancements were implemented in the iTIES through the duration of Phase II. The pumping sub-system was upgraded to include a booster pump, allowing for the effective transport of water to and through the iTIES. A drip chamber was also installed within the iTIES to remove gas bubbles from water, which may disrupt water flow and endanger organisms.

TASK 2: TESTING OF EARLY LIFE STAGE FISH

Early life stage fish were successfully used in numerous lab-based iTIE tests and field iTIES deployments, in both marine and freshwater applications. In addition to survival as an indicator of acute toxicity, several chronic toxicity endpoints were measurable in fish following 24- or 48-hour iTIE exposures, including teratogenicity and growth. Embryo-stage fish were ideal test organisms for iTIE experiments, due to their sensitivity, transportability, and low space requirements during exposure. Additional endpoints may be investigated for iTIE usage, including heart rate, olfaction, and genetic effects.

TASK 3: EXPANSION OF AVAILABLE SUBLETHAL CHRONIC ENDPOINTS IN INVERTEBRATE TEST ORGANISMS

Several sublethal chronic endpoints in invertebrate test organisms were verified for iTIE usage. AFDW was verified as a simple endpoint that was responsive to many toxicants. AChE-specific activity in amphipods was also effectively used to causally link organophosphate pesticides to organism toxicity in iTIE exposures. Results suggested the potential for other enzymatic bioassays to be used with the iTIES. Some reproductive endpoints including brood timing may have been influenced by stress induced by the iTIE procedure itself and thus warrant further study.

TASK 4: CONTINUATION OF RESIN OPTIMIZATION EFFORTS

Oasis WAX (Waters) was determined to be an optimal PFAS resin for use in iTIES experiments due to its strong survival and reproduction data compared to other candidate resins. Oasis WAX was effectively used in an iTIE experiment at Clark’s Marsh, allowing for the establishment of a stressor-toxicity linkage for PFAS.

Conditioning protocols were optimized for two crucial iTIE resins, Chelex and GAC. For use in freshwater deployments, Chelex had to be converted from its sodium form (as manufactured) to its calcium form to preclude impacts to water hardness and pH that may induce stress to organisms. GAC had to be conditioned with a period of aeration or other physical disturbance, which was found to mitigate water quality impacts to DO, pH, conductivity, and suspended solids. When properly conditioned, the two resins were powerful diagnostic treatments for iTIES experiments.

TASK 5: ADDITIONAL FIELD VERIFICATIONS IN MARINE AND FRESHWATER ENVIRONMENTS

1) The iTIES prototype was successfully field-tested at Third Sister Lake and Fleming Creek. The prototype configuration developed in this project phase is recommended for future iTIES designs. Biological results from the deployments indicated that both sites were relatively clean and may be designated as appropriate reference sites for environmental risk assessments in southeast Michigan.

2) The iTIES deployment at the Sexton and Kilfoil Drain provided some evidence of chemical toxicity, likely due to non-PFAS organic CoCs. However, the results of the deployment were confounded by low control group survival. Control group mortality was attributed to issues with fish culturing protocols, which were rectified in later iTIES experiments.

3) At Paleta Creek, the iTIES silicone-based oxygenation system effectively aerated porewater while decreasing dissolved sulfide concentrations. A high degree of chemical toxicity was detected, with widespread mortality in *A. bahia* larvae in all treatments after only 8 hours of exposure. *A. affinis* embryos exposed for 8 hours to unfractionated water also had extremely low survival (10%). The Oasis HLB and Oasis WAX treatment groups both had full survival, indicating that an organic CoC, likely pyrethroids, was the dominant stressor. The Chelex treatment group had an improved but still lower proportion of survival (40%), indicating that metals were a secondary cause of toxicity at the site. Additionally, *A. affinis* embryos exposed for 48 hours to vigorously aerated water samples experienced complete mortality, eliminating sulfides as a dominant stressor while underscoring the site's high degree of toxicity.

4) PFAS was confirmed as the dominant stressor at Clark's Marsh. For PFAS-sensitive *C. dilutus* larvae, the highest survival was observed in the Oasis WAX treatment group, associated with the lowest concentrations of PFAS. The zeolite treatment groups for both species also saw moderate survival, indicating that a chemical class targeted by the resin, such as ammonia, was another dominant stressor at the site. Finally, relationships were able to be established between chronic toxicity at the site and PFOS, copper, nickel, and zinc, indicating that heavy metals were potentially a source of stress at the site.

5) The dominant stressors at the Rouge River were found to be heavy metals and PFAS. *C. dilutus* larvae had the highest survival rate in the Oasis WAX treatment. *P. promelas* larvae, which were less sensitive to PFAS but more sensitive to heavy metals, had the highest survival rate in the Chelex treatment. Some CoC classes including polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls were not detected at the site, contrary to expectations.

TASK 6: DEVELOPMENT OF A DECISION-MAKING FRAMEWORK TO GUIDE ITIES IMPLEMENTATION

A decision-making framework was developed with five tiered steps to aid decision-making for remediation, recovery or site status projects:

1. First, an appropriate reference condition must be established.
2. It must be determined whether chemical or non-chemical stressors dominate at a site, including chronic toxicity and bioaccumulation potential.

3. Next, a Go/No-Go decision can be made on whether to conduct iTIE exposures to establish stressor-causality linkages and CoC ranks.
4. If successful weight-of-evidence evaluations have been conducted, the likely dominant stressor classes will have been verified.

5.0 IMPLICATIONS FOR FUTURE RESEARCH AND BENEFITS

The iTIES was an effective, durable, user-friendly, highly sensitive, and broadly applicable diagnostic tool that could be used to strengthen stressor-causality linkages and rank CoC classes at sites impacted by multiple stressors. The iTIES could be deployed diverlessly to sample sediment porewater with minimal surface water infiltration and gently aerate the porewater to prevent organism stress due to hypoxia. An array of available resins could be used to separate CoC classes including ammonia; heavy metals; nonpolar organics like organophosphates, PAHs, and pyrethroids; and polar organics like PFAS. Early life stage fish were compatible with iTIE testing, with teratogenicity and growth as highly sensitive endpoints for chronic toxicity. Other sub-chronic endpoints like AChE-specific activity were verified with the iTIES, indicating the potential for additional enzymatic bioassays to be applied to the testing technology. Data could be produced linking the presence of CoCs with toxicity, thus reducing personnel hours requirements compared with traditional testing protocols.

The progress made in Phase II continued to suggest that the iTIES should become a standard diagnostic assessment technology for incorporation into weight-of-evidence studies. After Tier 1 assessments suggest that chemicals may be causing toxicity at a site, the iTIES should be integrated as a Tier 2 or 3 level methodology to determine which CoC classes should be targeted in further study or remediation efforts. The potential remains for additional diagnostic resins, test organisms, and toxicity endpoints to be verified and applied for iTIES use.

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