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Preparation of Sediment Amended with Insensitive Munitions (IM) Compounds for Use in Toxicity Tests with Freshwater Invertebrates

Scientific Operating Procedure Series: Characterization of IMX Ecotoxicological Effects

Guilherme R. Lotufo

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Abstract

Updated methods for assessing the sediment toxicity of insensitive munitions (IM) compounds using freshwater benthic invertebrates are recommended by United States Army Corps of Engineers (USACE). A solvent-free method is used to spike sediment that is considered uncontaminated and suitable for use in whole sediment toxicity tests with IM formulations or their single constituents. Selection of sediment with low organic carbon and ammonia content is recommended. For each treatment, carrier solvent containing the appropriate mass of IM formulations, or one of their single constituents, is transferred into an open top jar and rolled on a horizontal rolling mill while the solvent is removed by evaporation. This leaves a coating of compound on the inner jar surface. Wet sediment is added to the jar which is then closed tightly. The sealed jar is rolled to abrade the compound from the jar walls and mix with the sediment.

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Contents

Abstract	ii
Preface	iv
Acronyms and Abbreviations	ii
1 Introduction.....	1
1.1 Background	1
1.2 Purpose	1
2 Materials.....	2
3 Procedural Overview.....	3
4 Reporting	6
References	7
Report Documentation Page	

Preface

This Scientific Operating Procedure (SOP) was developed under the Engineer Research Development Center - Environmental Quality and Technology (ERDC-EQT) Research Program project 398708 titled “Environmental Fate in the Life Cycle Analysis of New Materials.” The technical monitor was Dr. Elizabeth A. Ferguson.

The work was coordinated by the Environmental Processes and Risk Branch of the Environmental Processes Division (CEERD-EPR) at the U.S. Army Engineer Research and Development Center – Environmental Laboratory (ERDC-EL). Dr. William M. Nelson was Branch Chief, CEERD-EPR, Mr. Warren P. Lorentz was the Division Chief, CEERD-EP, and Dr. Elizabeth A. Ferguson was the Technical Director for Military Environmental Engineering and Sciences. The Deputy Director of ERDC-EL was Dr. Jack E. Davis and the Director was Dr. Ilker R. Adiguzel.

The Commander of ERDC was COL Ivan P. Beckman and the Director was Dr. David W. Pittman.

Acronyms and Abbreviations

Acronym	Meaning
DNAN	2,4-dinitroanisole
DoD	Department of Defense
EL	Environmental Laboratory
EPR	Environmental Process Division
EQT	Environmental Quality and Technology
ERDC	Engineer Research Development Center
IM	Insensitive Munitions
IMX-101	Mixture of DNAN, NTO and NQ
IMX-104	Mixture of DNAN, NTO, and RDX
L	Liter
mL	Milliliter
NTO	2-nitro-1,2,4-triazol-5-one
NQ	1-nitroguanidine
<i>p</i>	Percent Effect
ppt	Parts per thousand
SOP	Scientific Operating Procedure
TOC	Total Organic Content
USACE	U.S. Army Corps of Engineers
USEPA	U.S. Environmental Protection Agency

1 Introduction

The method for preparing sediment spiked with insensitive munitions (IM) compounds described herein was developed to provide a scientific operating procedure (SOP) to prepare contaminated solid-phase matrices for use in sediment toxicity testing with freshwater invertebrates as described in Lotufo and Farrar (2018). This approach is recommended to prepare sediments spiked with IM formulations, such as IMX-101 (a mixture of DNAN [2,4-dinitroanisole], NTO [2-nitro-1,2,4-triazol-5-one] and NQ [1-nitroguanidine]) and IMX-104 (a mixture of DNAN, NTO, and RDX) or with the individual constituents IM formulations.

1.1 Background

Spiking involves adding one or more chemicals to sediment for experimental purposes (U.S. Environmental Protection Agency [USEPA] 2000). Spiked sediments are used in toxicity tests to determine the effects of the spiked chemicals and their transformation products (if present) on test species. Spiking contaminants into sediment or soil using organic solvent carriers introduces additional organic carbon, extracts labile components, and alters the distribution of natural sediment organic matter (Northcott and Jones 2000). Therefore, to eliminate the disadvantages of solvent carriers, their direct addition should be avoided and shell coating methods should be used instead (USEPA 2000). Sufficient contact time between IM compounds and the sediment prior to conducting toxicity testing should be provided. Increased contact time, or aging, affects the chemical composition, bioavailability, and toxicity of organic compounds in soils and sediments, including munitions constituents (Northcott and Jones 2000; Conder et al. 2004; Kuperman et al. 2013; Lotufo et al. 2016). This SOP provides a method for spiking IM compounds with sediments that is solvent-free, and provides adequate equilibration time prior to use in toxicity testing.

1.2 Purpose

The purpose of this document is to provide a detailed method to prepare contaminated sediments for use in sediment toxicity testing. Procedures for using sediments spiked with IM formulation or a single IM constituent to evaluate their toxicity to the freshwater amphipod *Hyalella azteca* and the midge *Chironomus dilutus* is provided in Lotufo and Farrar (2018).

2 Materials

This protocol utilizes the following materials, reagents, and equipment:

- Uncontaminated freshwater sediment
- One-gallon amber glass jars
- 25-mL beakers
- Organic solvent carrier (e.g., acetone)
- Pasteur pipettes
- Horizontal rolling mill
- Plastic paraffin film (e.g., Parafilm®)
- Pre-cleaned glass jars (e.g., 125 mL) for holding sediment for chemical analysis
- Stainless steel spoons

3 Procedural Overview

A solvent-free method is used to spike uncontaminated sediment suitable for use in whole sediment toxicity tests (Lotufo and Farrar 2018) with IM formulations or their single constituents. The selected sediment should be essentially free of contaminants and suitable for use as a control sediment (i.e., to routinely assess the acceptability of a toxicity test). Any contaminants in a control sediment are thought to originate from the global spread of pollutants and do not reflect any substantial input from local or nonpoint sources (USEPA 2000). Whole sediment toxicity tests with IM formulations are preferably conducted as static (no overlying water renewal) exposures (Lotufo and Farrar 2018). Because ammonia may build up in the porewater and in the overlying water during the exposure period, the sediment selected for assessing the toxicity of IM compounds in sediment exposures should have a low concentration of ammonia, so that the concentration of unionized ammonia (as ammoniacal nitrogen, $\text{NH}_3\text{-N}$) in the overlying water of exposure beakers does not exceed 0.2 mg/L during 10-day static tests (Environment Canada 2013).

In addition, the use of sediments that cause relatively low degradation of parent compounds is preferable. A predominantly sandy sediment with a low total organic carbon content (TOC) (0.1 %) yielded relatively low transformation of IM compounds (measured concentration of parent compound DNAN, NTO, NQ and RDX corresponding to ≥ 57 % the target concentration).¹ For each treatment, carrier solvent containing the appropriate mass of IM formulations, or one of their single constituents, is transferred into an open top jar and rolled on a horizontal rolling mill while the solvent is removed by evaporation. This leaves a coating of compound on the inner-jar surface. Wet sediment is added to the jar which is then closed tightly. The sealed jar is rolled to abrade the compound from the jar walls and mix with the sediment. Detailed procedures are provided in this SOP.

When performing this spiking procedure for the first time with an uncontaminated sediment, a preliminary experiment should be conducted using the spiking procedure described in this SOP to determine if excessive transformation occurs during the mixing period. If the fraction

¹ Lotufo, Guilherme R., Chappell Pornsawan, and Michael G. Jung (In Preparation) Toxicity of insensitive munitions formulations and single constituents to freshwater invertebrates in exposure to spiked sediment. Environmental Toxicology and Chemistry.

of the parent compound that transforms to known or non-identified compounds is considered excessive, (e.g., less than 70% of the spiked amount is present at termination of the mixing period) a different uncontaminated sediment with a lower TOC should be selected and evaluated.

1. Establish the target concentrations for IM formulations or one of their single constituents in the sediment treatments that will be used in whole sediment toxicity tests.
2. Obtain sufficient mass of a freshwater uncontaminated sediment suitable for use as a control sediment for toxicity tests conducted with benthic invertebrates. According to Lotufo and Farrar (2018), excess of 0.6 L of sediment is necessary for loading six replicate beakers for a whole-sediment toxicity test. To ensure sufficient sediment for toxicity testing and for chemical analysis, a minimum of 1 L of spiked sediment for each treatment of a toxicity test should be prepared.
3. Sieve the uncontaminated sediment selected for spiking if removal of debris, gravel, or indigenous organisms is necessary (USEPA 2001).
4. Determine the moisture content of the sediment in triplicate following procedures described in USEPA (2000).
5. Obtain sufficient mass of the IM formulations or one of their single constituents that will be spiked to uncontaminated sediment.
6. Obtain pre-cleaned, one-gallon amber glass jars (or clear-glass jars wrapped in aluminum foil), one jar per treatment.
7. Prepare dosing solutions by dissolving the appropriate mass of the test chemical or chemical mixture in the appropriate carrier solvent (e.g., acetone). Add the mass of chemical to a 25 mL beaker. Add sufficient organic solvent (e.g., 10 mL) to fully dissolve the test chemical or chemical mixture.
8. Transfer each dosing solutions into pre-labeled open top jars. Rinse the 25 mL beaker with clean solvent and place into the open top jar. Place jars on a horizontal rolling mill and roll the jars until all the solvent is removed by evaporation, leaving a coating of compound on the inner surface of the jars. This process can be accelerated by gently blowing air into the jars.
9. Add wet sediment to each jar. Tightly close each jar with its lid and seal with paraffin film (e.g., Parafilm®).
10. Roll the jars for 14 days at approximately 15 rpm. Optimally wetted, individual substrate particles adhere to each other and to the wall of the revolving jar until they tumble down the surface of the substrate mass.

Dechlorinated tap water may be added to the substrate before rolling to adjust the sediment-to-water ratio for optimal mixing.

11. At termination of the rolling period, shake the sediment in each jar to integrate the sediment that adhered to the walls to the homogenized sediment. Add the spiked sediment to test beakers set up for toxicity testing (Lotufo and Farrar 2018). Alternatively, the jars can be stored overnight or longer, if desired, in the dark at room temperature or refrigerated (e.g., 4° C) for equilibration depending on the study objectives. After equilibration and prior to distributing the sample to test beakers, shake the jars and or stir the sediment with a stainless steel spoon to integrate overlying water formed during settling into the sediment.
12. Obtain sediment for chemical analysis while distributing sediment to test beakers. Transfer sediment from each treatment to the appropriate pre-cleaned glass jars and keep samples at 4 °C in the dark until analysis is conducted. Consult the analytical laboratory for sample mass and holding time.

4 Reporting

Table sheets should be used to record daily activities and data derived from measurement (e.g., moisture content).

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