

Investigating Sea-Dumped Munitions in the Pacific Hawaii Undersea Munitions Assessment (HUMMA) Project - PHASE I

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Introduction

According to historical records, between the years 1933 and 1946 the United States Armed Forces disposed of chemical munitions and containers of bulk chemical warfare materials (CWM) off O'ahu, Hawaii, according to the accepted maritime disposal procedures of the time. The specific chemical agents disposed include the blister agents Mustard (HD) and Lewisite (L). The Department of Defense (DoD) ended its practice of sea disposal (Fig.1 and 2) of military munitions and CWM in 1970 and disposal at sea was generally prohibited by Congress in 1972 with the passage of the Marine Protection, Research and Sanctuaries Act.

In addition to providing on-site analysis of water and sediment, ECBC was tasked to provide a method of analysis to determine the levels (if any) of CWM (HD, Lewisite (L), and the HD break-down products 1,4-Dithiane and 1,4-Thioxane) in biota that would be harvested in the HUMMA study area. The experimental process, Method



Figure 1. Disposal of Munitions at Sea (photo courtesy of the National Archives and Records Administration).



Figure 2. Modern Day Munition Located in the HUMMA Study Area, note similarity to items in Fig. 1 (photo courtesy of Hawaii Undersea Research Laboratory).



Figure 3. University of Hawaii's (UH) Research Vessel *Kilo Moana*.

Detection Limit (MDL) study, and the analytical results for the samples collected in the spring and fall of 2009 aboard the *Kilo Moana* (Fig. 3) are presented in this poster.

Materials and Methods

MDL Fish Tissue Sample Preparation. To imitate the fish sample matrix Onaga (Ruby Snapper), a whole frozen red snapper was purchased from a commercial supermarket. After thawing, the fillets were removed from the fish utilizing a filleting knife. The fillets were cut into pieces and then pulverized with a mortar and pestle into a single homogenous sample. Due to the similarity in sample matrix of fish tissue and shrimp tissue a separate MDL was not performed for the shrimp. This decision was supported by the results of the Matrix Spike/Matrix Spike Duplicate (MS/MSD) that showed adequate recovery with minimal interference.

HUMMA Sample Collection. From late April through early May 2009, sampling of fish and shrimp in or near the HUMMA study area took place. The samples were packaged and shipped to ECBC for CWM analysis. The fish tissues samples analyzed included fillets only from a Large Onaga, whereas shrimp tissues analyzed included tails only, to be reflective of local consumption habits (Fig. 4). Fish fillets were of sufficient mass to represent a unique sample; however, in some cases, two or more shrimp tails were combined to achieve the minimum sample mass required. In these instances, shrimp of roughly the same size and from the same shrimp trap were combined. Samples were sent directly to Columbia Analytical Services, where they were processed and analyzed for energetics and metals. Columbia Analytical Services also prepared a subset of extract that was sent to ECBC to be analyzed for HD, Lewisite and the degradation products 1,4-Dithiane and 1,4-Thioxane (The University of Hawaii at Manoa, 2010).

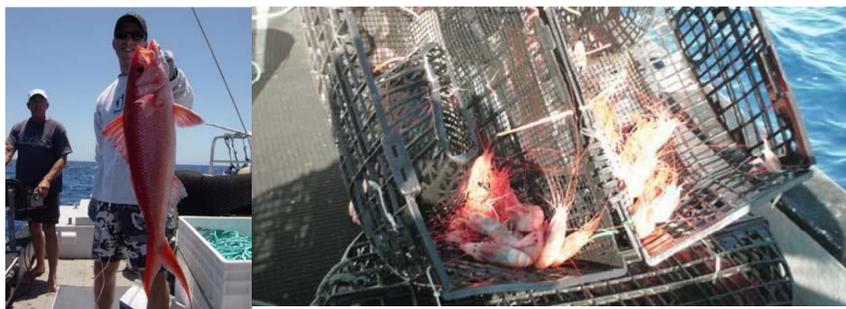


Figure 4. LEFT: Large Onaga caught onboard *Red Raven* during HUMMA biota sampling. RIGHT: Shrimp samples caught during biota collection.

Abstract

In 2008 the Environmental Monitoring Branch (EML) of the U.S. Army Edgewood Chemical and Biological Center (ECBC) was tasked by the Office of the Deputy Assistant Secretary of the Army for Environment, Safety and Occupational Health (ODASA-ESOH) to provide chemical agent safety and analytical support to the University of Hawaii at Manoa (UH). UH was subcontractor to Concurrent Technologies Corporation (CTC) under Task No.: 0496 of Contract W74V8H-04-D-0005 issued by the National Defense Center for Energy and Environment (NDCEE). The task for the Hawai'i Undersea Military Munitions Assessment (HUMMA) was to evaluate whether the munitions have the capability to significantly impact human health (specifically in regard to the introduction into the food chain) and the environment. From a broader perspective, HUMMA's objective was to develop and demonstrate cost-efficient and effective methodologies for surveying and sampling historic munitions sea disposal sites. The EML developed a procedure and conducted a Method Detection Limit (MDL) study for the analysis of Chemical Warfare Materials (CWM) in fish tissue in support of the HUMMA project. The scope of ECBC's study included developing new methodology to detect, accurately quantitate, and find the Limit of Quantitation (LOQ) for detecting the CWM Lewisite, Mustard, and its breakdown products 1,4-Dithiane and 1,4-Thioxane; and extracting and analyzing samples collected in the HUMMA assessment area for CWM.

Results

A total of forty eight tissue samples were received, extracted, and analyzed by GC/MS for the HUMMA project (Tables 1, 2). All of the samples were analyzed in Selective Ion Mode. By comparing the Extracted Ion Chromatogram of the matrix spike for EML091524 and sample EML091524, it can be concluded that:

- There were no agents detected above the LOQ
- The instrument would be capable of detecting the agents if they were in the sample
- The remaining samples were all clear for HD, 1,4-Dithiane, and 1,4-Thioxane to the Laboratory LOQ.

Table 1. Sample Results for HUMMA Shrimp Samples

ECBC Sample Number	HUMMA Sample Number	Results (µg/Kg = ppb)			
		HD	Lewisite	1,4-Dithiane	1,4-Thioxane
EML091524	HUM001S	<10	<100	<100	<100
EML091525	HUM002S	<10	<100	<100	<100
EML091526	HUM003S	<10	<100	<100	<100
EML091527	HUM004S	<10	<100	<100	<100
EML091528	HUM005S	<10	<100	<100	<100
EML091529	HUM006S	<10	<100	<100	<100
EML091530	HUM007S	<10	<100	<100	<100
EML091531	HUM008S	<10	<100	<100	<100
EML091532	HUM009S	<10	<100	<100	<100
EML091533	HUM010S	<10	<100	<100	<100
EML091534	HUM011S	<10	<100	<100	<100
EML091535	HUM012S	<10	<100	<100	<100
EML091536	HUM013S	<10	<100	<100	<100
EML091537	HUM014S	<10	<100	<100	<100
EML091538	HUM018S	<10	<100	<100	<100
EML091539	HUM019S	<10	<100	<100	<100
EML091540	HUM021S	<10	<100	<100	<100
EML091541	HUM024S	<10	<100	<100	<100
EML091542	HUM025S	<10	<100	<100	<100
EML091543	HUM029S	<10	<100	<100	<100
EML091544	HUM030S	<10	<100	<100	<100
EML093424	HUM015S	<10	<100	<100	<100
EML093425	HUM016S	<10	<100	<100	<100
EML093426	HUM017S	<10	<100	<100	<100
EML093427	HUM020S	<10	<100	<100	<100
EML093428	HUM022S	<10	<100	<100	<100
EML093429	HUM023S	<10	<100	<100	<100
EML093430	HUM026S	<10	<100	<100	<100
EML093434	HUM027S	<10	<100	<100	<100
EML093432	HUM028S	<10	<100	<100	<100

Table 2. Sample Results for HUMMA Fish Samples.

ECBC Sample Number	HUMMA Sample Number	Results (µg/Kg = ppb)			
		HD	Lewisite	1,4-Dithiane	1,4-Thioxane
EML091545	HUM001F	<10	<100	<100	<100
EML091546	HUM002F	<10	<100	<100	<100
EML091547	HUM003F	<10	<100	<100	<100
EML091548	HUM004F	<10	<100	<100	<100
EML091549	HUM005F	<10	<100	<100	<100
EML091550	HUM006F	<10	<100	<100	<100
EML091551	HUM007F	<10	<100	<100	<100
EML091552	HUM008F	<10	<100	<100	<100
EML091553	HUM009F	<10	<100	<100	<100
EML091554	HUM010F	<10	<100	<100	<100
EML091555	HUM011F	<10	<100	<100	<100
EML091556	HUM012F	<10	<100	<100	<100
EML091557	HUM013F	<10	<100	<100	<100
EML091558	HUM014F	<10	<100	<100	<100
EML091559	HUM015F	<10	<100	<100	<100
EML091560	HUM016F	<10	<100	<100	<100
EML091561	HUM017F	<10	<100	<100	<100
EML091562	HUM018F	<10	<100	<100	<100

Materials and Methods (cont'd)

HUMMA Sample Extraction. The HUMMA fish tissue and shrimp samples arrived at ECBC in May and September of 2009. They were received frozen and already pulverized and were stored at -16°C prior to extraction. The samples were removed from the freezer and equilibrated to room temperature. Method blank and lab control standards were prepared by weighing 2 g of commercially-purchased red snapper in a 16mm test tube. The MS, MSD, and HUMMA samples were prepared by pulverizing 2 g of HUMMA fillet/shrimp in a 16 mm test tube (Fig. 5). One hundred microliters of the surrogate/internal standard spike Bromofluorobenzene /Hexachlorobenzene (BFB/HCB at 5000 µg/L each) was added to each 16 mm tube. One hundred microliters of each matrix spiking standard (5000 µg/L for 1,4-Dithiane, 1,4-Thioxane, and Lewisite; 500 µg/L for HD) was added to the lab control standards, MS, and MSD, and 1.9 mL of Dichloromethane containing 0.1% β-mercaptoethanol (BME) was added to the test tube in excess to derivatize lewisite and its hydrolysis products CVAO and CVAA for analysis by GC/MS. The test tubes were vortexed for approximately 1 min and then centrifuged at 5000 RPM for 10 min. The organic extract layer was then filtered through a 0.4 µm Polytetrafluoroethylene (PTFE) filter into a 2 mL vial for analysis. Samples were directly injected onto GC/MS (Fig. 7) using procedures specified in ECBC IOP MT-8 Revision 5: *Analysis of Chemical Warfare Agents in Extracts using a Gas Chromatograph/Mass Spectrometer System*.



Figure 5. HUMMA fish tissue sample (left); GC/MS (right).

Discussion

ECBC was tasked with developing an analytical approach to detecting CWM and agent breakdown products in fish tissue. Our laboratory was able to develop a method to detect and accurately quantify the amount of 1,4-Dithiane, 1,4-Thioxane, Mustard and Lewisite in fish tissue utilizing GC/MS. ECBC was able to analyze, validate, and generate results utilizing the procedures developed in the method detection limit study. The data generated during the project was used to determine if the target chemical agents and breakdown products were present below the LOQ of 100 ppb for 1,4-Dithiane, 1,4-Thioxane, and L, and 10 ppb for HD. This analysis demonstrates that there was no direct contamination present in the biota samples collected; however, any fish metabolism of agent cannot be determined from this method. This study will serve as the basis for any future ECBC efforts to extract and analyze biota samples in support of similar projects.