



# Abstract Book

## DAIMON Final Conference

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## A2.1 Information systems of dumped chemical and conventional munitions

Bartłomiej Pączek

Polish Naval Academy, Gdynia, Poland

During the DAIMON project, multiple ship cruises have been conducted in order to collect research data on chemical and conventional munitions, dumped in the Baltic Sea. The research covered, in parallel, chemical and biological analyzes of environmental samples, as well as archives research and compilation of available paper catalogs of munition pieces. The results of the research have been publically presented in the form of Geographic Information System (GIS), that allows users to browse not only locations of sampling, but also results of analyzes conducted. In order to broaden public availability of information on chemical and conventional weapons dumped in the Baltic Sea, the GIS system has been coupled with an on-line catalog of munitions, aimed to deliver the tool that will help to identify munition pieces that may be encountered in the Baltic Sea. Main functionalities of the catalog allow users to list munition pieces based on its features, and bring detailed information on possible threats connected with contact with warfare agents, they may contain.

## A2.1 The rate of corrosion of munitions dumped in the Baltic Sea

Jacek Fabisiak and Edyta Lonska

Polish Naval Academy, Gdynia, Poland

Within the "DAIMON" project, was carried out a coupon corrosion study on corrosion rate of metallic construction materials of dumped chemical munitions and containers in which chemical warfare agents (CWA) were stored. Materials that were examined in the form of coupons, were obtained from two original elements from the WWII (artillery shell and barrel cover), but also from materials currently manufactured (steel sheets), however with a composition similar to the chemical composition corresponding to the construction materials of ammunition produced before and during WW II.

The research on the rate of corrosive dissolution was carried out in two parallel experiments: *ex-situ* and *in-situ*. Analysis of the obtained rates of corrosion in the *ex-situ* experiment indicates that the corrosion rate ranges from  $0.0025 \text{ mm} \cdot \text{year}^{-1}$  to  $0.0926 \text{ mm} \cdot \text{year}^{-1}$  and depends mainly on the type of corrosion material and collection site of bottom sediments. Results indicates, that the corrosion rate varies with the region from which the environmental material was collected. Thus, the highest corrosion rates are observed in the area of the Pomeranian Bay and in the Kolberger Heide region, smaller in the area of the Bornholm Deep, even smaller in the Little Belt and the Gdańsk Deep, while the smallest in the Slupsk Furrow area. During *in-situ* experiments, water was main corrosive environment. The obtained corrosion rates differ from those determined during the *ex-situ* experiment. And they range from  $0.0243 \text{ mm} \cdot \text{year}^{-1}$  in the Gdańsk Deep to  $0.1210 \text{ mm} \cdot \text{year}^{-1}$  in the Bornholm Deep.

On the basis of *in-situ* experiment, assuming 70 years of retention of the munitions in the marine environment, it can be concluded that the thickness of barrels that are not covered by the sediment has decreased by approx 1.7 mm to 6 mm, therefore nowadays, regardless areas where they were dumped and environmental conditions prevailing in these areas, the barrel are completely destroyed and unsealed, and the CWA that they were filled with have already been released into the marine environment (the original thickness of the barrels was about 1.5 mm). In the case of artillery shells laying on the bottom surface and not covered with sediments, the thickness of the shell has decreased by 5.7 mm to 8.5 mm, therefore it can be assumed that they are corroded, but they can still be tight (as the thickness of the artillery shell varies from 10 up to 30 mm). Whereas in the case of aerial bombs not covered with bottom sediment, their thickness decreased by 1.3 to 7.5 mm, therefore bombs that were not covered with sediment are now completely destroyed and unsealed and CWA probably have been released into the environment (as the shell thickness varies from 1.5 to 3 mm).

However, in the case of materials immersed in bottom sediments, on the basis of *ex-situ* experiment, it can be determined that after 70 years of the deposition of munitions in the marine environment that the thickness of barrels covered with sediment decreased by 0.6 mm to 4 mm depending on the area of dumping. Therefore, regardless of the storage area of the barrels and environmental conditions, including the thickness of bottom sediments coverage, it should be considered that the barrels are completely destroyed and unsealed, and the CWA that they were filled with have already been released into the environment. In the case of artillery shells (with original body thickness 10 - 30 mm) covered with sediment, the thickness of their shells decreased by approx 1.1mm to 1.3mm, hence it can be assumed that currently this type of ammunition is still in very good condition and CWA (explosives) contained in it have not yet been released into the marine environment. In the case of aerial bombs covered with sediment the thickness of their

bodies decreased by 0.1 mm to 6 mm. Therefore, bombs covered with a thin layer of sediment may already be destroyed or heavily corroded, so that in case of bomb recovery, bomb may break. However, in the case of covering them with a thick layer of sediments, in regions with a reduced activity of corrosive factors (e.g. the activity of microorganisms) also bombs with little rust and still containing CWA can be found.

## A2.1 Munition Payload Identification via Neutron Activation Analysis

Hannes Vainionpää<sup>1</sup>, Markku Paaso<sup>1</sup>, Sakari Ihantola<sup>1</sup>, Pekka Salmi<sup>1</sup>, Paula Vanninen<sup>2</sup>,  
Martin Soderstrom<sup>2</sup>, Hanna Niemikoski<sup>2</sup>

<sup>1</sup>NeutronGate Oy, Riihimäki; Finland

<sup>2</sup>Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN), Helsinki, Finland

There exists a large amount unexploded ordnance (UXO) dumped on the seas. These munitions consists mainly of High Explosives (HE) and Chemical Weapons Agents (CWA). These munitions pose multiple risk modalities to the surrounding environment and human activities. The identification and evaluation of these munitions has been a challenge. The general vicinity of a CWA (much of the Baltic sea) may be identified using sediment and water samples. However, as of today, no cost-effective method exists to identify and characterize individual munitions lying on the seabed. Disposing of CWA munition is an expensive and dangerous task, as handling of CWA munitions requires special care and they may not be disposed in situ. Today, almost every munition has to be handled as if it was CWA. Because of this the inability to distinguish the various munitions and their conditions on the spot makes larger scale clearing economically infeasible. With proper identification and evaluation, the HE -munitions may be destroyed at the site via controlled explosion and the CWA:s may be handled in appropriate manner.

We have demonstrated a novel method to solve this classification problem. Using elemental analysis based in neutron activation (NAA), we are able to define the elemental composition of the munition from a distance in an accurate and safe manner. We are able to identify the type of a munition fairly quickly, and with longer measurements it is further possible to determine the corrosion level and possibly the existence and amount of leakage. This data is critical for economic decision making regarding the clearing of the most hazardous objects. With this data it is possible to make informed decision regarding the questions: When and what actions are required and which actions are reasonable and efficient? Also concept of a desirable operational system was envisioned during DAIMON project.



## A2.2 Leakage rate of the nerve agent tabun from sea-dumped ammunition

John Aa Tørnes, Thomas Vik and Tomas Tungen Kjellstrøm

Norwegian Defence Research Establishment (FFI, Kjeller, Norway)

Large amounts of chemical ammunitions were dumped at sea in many locations worldwide just after the Second World War. Much of the German chemical ammunitions were loaded on to condemned ships and sunk in the deepest part of Skagerrak at 600-700 meters depth. Ammunition dumped at sea will corrode over time and the content will eventually be released to the environment. Some of the sunken munitions contain the nerve agent tabun. This paper describes laboratory experiments carried out to measure the leakage rate of tabun from a corroded aerial bomb and a simple mathematical model to estimate leakage rates. A model of a fairly common chemical bomb, the KC250 aerial bomb has been used in a laboratory experiment to simulate the leakage of tabun. Tabun will hydrolyse to less toxic compounds. The effect of hydrolysis on the amount of tabun that will leak out from the bomb is included in the mathematical model. If a hole develops on top of the bomb, very little tabun will leak out since tabun is denser than water, instead the nerve agent will hydrolyse inside the bomb and light hydrolysis products will exit the bomb. If the hole develops on the bottom half of the bomb, some of the tabun will leak out. However, hydrolysis will still take place, and it is estimated that for a hole of about  $10 \text{ cm}^2$ , about half of the original content of tabun in the bomb will be released into the sea during four hours, while the rest of the agent will hydrolyse inside the bomb.

## A2.3 Contamination of marine sediments with arsenic and heavy metals originating from dumped munitions

Marta Szubska, Grzegorz Siedlewicz, Ewa Korejwo, Jacek Beldowski  
Institute of Oceanology of the Polish Academy of Sciences (IOPAN), Sopot, Poland

Since chemical and conventional munitions consist of various material and substances, their dumpsites may occur as a local anthropogenic source of arsenic and heavy metals to the environment in close vicinity to munition dumpsites. During the DAIMON project, monitoring of arsenic and heavy metals was performed in the areas of military ordnance dumping locations. An elemental analysis was performed with the use of handheld X-Ray Fluorescence Spectrometer S1 TITAN with calibration mode designed for geological and soil samples. Basing on the comparison of results from reference materials analyzed with mobile XRF and standard laboratory AAS and ICP-MS the accuracy of analysis is acceptable, therefore it is assumed as a useful tool for geochemical measurements of marine bottom sediments. Main focus was given to the concentrations of arsenic (as the compound of arsenic-based chemical warfare agents - CWA) and lead (as lead azide was one of the compound widely used as an initiating material in various military ordnance), iron and manganese (which have significant influence on elements biogeochemistry in sediments) and heavy metals: chromium, nickel, zinc. In general concentrations of measured elements were typical for fine-grained sediments of the Baltic Sea, however several slightly elevated concentrations of arsenic were measured in the dumpsites, in comparison with geochemical background.

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### A2.3 Concentration of methylmercury in marine sediments collected in munition dumpsite

Grzegorz Siedlewicz, Marta Szubska, Ewa Korejwo, Jacek Beldowski  
Institute of Oceanology of the Polish Academy of Sciences (IOPAN), Sopot, Poland

Methylmercury (MeHg) is the most toxic and dangerous form of mercury occurring in the environment. MeHg is highly bioaccumulative in organisms and undergoes biomagnification via the food chain. In military ordnance dumpsites higher concentration of mercury are expected, as mercury fulminate was used as a popular primer explosive in detonators of munitions. In most of the munition dumpsites located in the Baltic Sea, environmental conditions promote methylation processes: anoxic conditions, presence of sulfate-reducing bacteria, presence of organic matter and no UV radiation. During the DAIMON project the analytical procedure was intended for determination of MeHg concentrations in the marine sediments. Results from the environmental samples from munition dumpsites located in the Baltic and North Sea show various concentrations of MeHg from MDL to hundreds  $\text{pg g}^{-1}$  d.w.

The research work was funded by the European Union (European Regional Development Fund) under the Interreg Baltic Sea Region Programme 2014-2020, project #R013 DAIMON, and supported with co-financing by the Ministry of Science and Higher Education from the 2016-2019 science funding allocated for the implementation of international co-financed project W81/INTERREG BSR/2016.

## A2.3 Sea-dumped ammunition as a source of mercury to the Baltic Sea sediments

Jacek Bełdowski<sup>1</sup>, Marta Szubska<sup>1</sup>, Grzegorz Siedlewicz<sup>1</sup>, Ewa Korejwo<sup>1</sup>, Miłosz Grabowski<sup>1</sup>, Magdalena Bełdowska<sup>2</sup>, Jacek Fabisiak<sup>3</sup>, Edyta Łońska<sup>3</sup>, Mateusz Szala<sup>4</sup>, Janusz Pempkowiak<sup>1</sup>

<sup>1</sup>Institute of Oceanology of the Polish Academy of Sciences (IOPAN), Sopot, Poland

<sup>2</sup>University of Gdańsk, Gdynia, Poland

<sup>3</sup>Polish Naval Academy, Gdynia, Poland

<sup>4</sup>Military University of Technology, Warsaw, Poland

After World War II, as a part of Germany's demilitarization, up to 385,000 tons of munition were sunk in the Baltic Sea. Object containing various dangerous substances – chemical Warfare Agents (CWA) - and explosives which can affect a marine environment. Some of those compounds, like mercury fulminate (a popular explosive primer), can be an additional local source of mercury in the dumping areas. Unfortunately there is a lack of information on how dumped munition impacts the mercury levels in Baltic Sea sediments. The aim of this study was to answer the question if those military objects can be assumed as point-sources of mercury to marine environment and to verify if mercury in those areas can originate from mercury fulminate. Concentration of  $Hg_{tot}$  in samples collected from conventional (Kolberger Heide) and chemical (Bornholm Deep) munition was characterized with high variability. However an increase of  $Hg_{tot}$  concentrations was observed with a decreasing distance from particular objects. In two cases, speciation analysis of sediments from Kolberger Heide proves that mercury originates from the mercury fulminate. Carried investigation shows that the impact of munition dumpsites as a local point source of mercury is probable but hard to evaluate. Also, further detailed studies should be conducted to assess the distribution of different forms of mercury in munition dumpsites more accurately.

The research work was fund by the European Union (European Regional Development Fund) under the Interreg Baltic Sea Region Programme 2014-2020, project #R013 DAIMON, and supported with co-financing by the Ministry of Science and Higher Education from the 2016-2019 science funding allocated for the implementation of international co-financed project W81/INTERREG BSR/2016.

## A2.5 Toxic effects of dissolved TNT on the Baltic mussels (*Mytilus* spp.): first results from lab exposure studies

Matthias Brenner<sup>1</sup>, Romina Schuster<sup>1</sup>, Jennifer S. Strehse<sup>2</sup>, Aino Ahvo<sup>3</sup>, Edmund Maser<sup>2</sup>, Ulf Bickmeyer<sup>1</sup>, Kari K. Lehtonen<sup>3</sup>

<sup>1</sup>Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Bremerhaven, Germany

<sup>2</sup>Institute of Toxicology and Pharmacology for Natural Scientists, University Medical School, Kiel, Germany

<sup>3</sup>Finnish Environment Institute (SYKE), Helsinki, Finland

During and after the WWs, large amounts of chemical weapons and conventional munitions stored on German territory were dumped in the Baltic Sea by order of the allied forces or German administration. In addition of being a cheap method of disposal, the belief was that the vast amounts of waters in the oceans would neutralize and absorb the dangerous substances. Nowadays, dumped warfare agents are posing a growing concern for the marine environment since dangerous contents are leaking from corroding metal shells and pollute ambient sediments and water. Both, chemical and conventional warfare agents and their degradation products have been detected in noticeable concentrations in sediments at the major dumping sites in the Baltic Sea.

However, the knowledge about dissolved explosives on the health of marine organisms is scarce. Even basic toxicity values such as lethal TNT concentrations are missing in literature for most organisms. Therefore, we conducted blue mussel exposure experiments in the lab to assess environmental impact of dissolved TNT. Biomarkers representing different biological functions including e.g. antioxidant defence, biotransformation, lysosomal membrane stability, cellular energy allocation and condition index were investigated. Moreover, tissue concentrations of TNT and its degradation products were analysed and correlated with the biological effects.

First results revealed that mussels show clear reactions of avoidance and negative biological effects when they are exposed to dissolved TNT under lab conditions. For example, shell closure, a simple mechanism to avoid any unfavourable conditions, leads to long exposure times under high concentrations of toxic compounds until lethal doses are reached. In contrast, concentrations of dissolved TNT where no negative effects on mussel's health were detectable were comparably low. Thus clearly demonstrating the overall toxicity of TNT for marine organisms.

## A2.5 A novel method for detection, identification and quantification of explosives compounds in fish

Nadine I. Goldenstein, Ulrike Machulik and Ulrike Kammann

Thünen Institute of Fisheries Ecology (TI-FI), Bremerhaven, Germany

Trinitrotoluene (TNT) as the most popular explosive substance in dumped ammunition at the seafloor is subject to various biotic and abiotic transformation processes in the marine environment. Nevertheless, other substances as Hexogen (RDX) and Octogen (HMX) are found alongside TNT. Since the derivatives of TNT are known to cause biological effects distinct from their precursor, the sensitive detection, identification and quantification of explosives compounds and their metabolites is of utmost importance for risk evaluation and management for dumped munitions.

To analyse explosives and their metabolites, we developed a novel method, based on liquid-liquid extraction of fish body fluids and tissue. Analysis of the extracts was established on an Agilent Technologies High Performance Liquid Chromatograph (HPLC) equipped with a Thermo Fisher Acclaim™ Explosives E2 LC column coupled to an AB Sciex Triple Quadrupole Mass Spectrometer (QQQ-MS), operated in negative atmospheric pressure ionization (APCI neg) mode. The highly sensitive quantitative detection of known explosives compounds and derivatives is achieved via Multiple Reaction Monitoring (MRM), simultaneously recording an Enhanced Mass Spectra (EMS) Scan for qualitative spectral information. In addition, the samples are being scanned for unknown nitro-containing components that might represent key-metabolites in the degradation of nitro-aromatic explosives. For identification of unknowns, Neutral-Loss (NL) scans detect the characteristic loss of a nitro-group in syncopation with highly sensitive Enhanced Product Ion (EPI) scans providing structural information based on sensitive mass spectra of the nitro-containing precursors.

With this setup, we provide an easily applicable method for quantification and identification of explosives compounds in fish, accumulating due to release of toxic nitro-aromatics from dumped ammunition. The analysis of metabolites, in addition to molecules of TNT, HMX and RDX, strongly enlarges our analytical window and hence, our knowledge on the fate of explosives in the environment. The methodological setup presented here can, therefore, enable in-depth future studies and conclusive monitoring activities on fish from munition dumpsites.

## A2.5 DAIMON Toolbox for the assessment of marine munitions impact on biota

Thomas Lang<sup>1</sup>, Ulrike Kammann<sup>1</sup>, Matthias Brenner<sup>2</sup>, Kari Lehtonen<sup>3</sup>, Paula Vanninen<sup>4</sup>

<sup>1</sup>Thünen Institute of Fisheries Ecology (TI-FI), Bremerhaven, Germany

<sup>2</sup>Alfred Wegener Institute for Polar and Marine Research (AWI), Bremerhaven, Germany

<sup>3</sup>Finnish Environment Institute (SYKE), Helsinki, Finland

<sup>4</sup>Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN), Helsinki, Finland

The DAIMON Toolbox provides a concept and strategy for analyzing and assessing ecological risks associated with dumped chemical or conventional munitions in marine ecosystems. Its major components are recommendations and structured methodological guidelines (method leaflets) for chemical and biological techniques to be applied to measure the release of harmful substances into the environment as well as their effects on marine biota. The application of the Toolbox is based on two consecutive tiers, a Screening Study for a first assessment and a following Detailed Study. The design of these studies and the methods applied depend on risk scenario to be analysed and assessed. For the Screening Study, a selected number of robust and easy-to-apply methods to measure chemical munitions compounds in the environment as well as their *in situ* biological effects is recommended. The Detailed Study is carried out if more specific information is needed and, thus, constitutes an extension of the Screening Study. It comprises of a broader set of methods and may include other approaches such as bioassays and laboratory toxicity studies. The results of both the Screening Study and the Detailed Study form the basis of risk assessments, the results of which are fed into the DAIMON Decision Support System.

## A2.5 Ecological impact of sea dumped chemical warfare on Baltic Sea ecosystem

Michał Czub<sup>1</sup>, Lech Kotwicki<sup>1</sup>, Jacek Bełdowski<sup>1</sup>, Stanisław Popiel<sup>2</sup>, Jakub Nawala<sup>2</sup>, Tomasz Brzeziński, Piotr Maszczyk<sup>3</sup>

<sup>1</sup>Institute of Oceanology of the Polish Academy of Sciences (IOPAN), Sopot, Poland

<sup>2</sup>Military University of Technology (MUT), Warsaw, Poland

<sup>3</sup>University of Warsaw, Warsaw (UW), Warsaw, Poland

In the middle of the XXth century, soon after the end of the WW II, the Baltic Sea ecosystem started to serve as a dumpsite for 40 000 tons of unused Chemical Warfare. Back then sea dumping was a common practice, therefore several offshore areas were selected, where munition loads were later sunk, making it taboo due to its problematic geopolitical background. It was not until the first decade of XXIst century, when problematic questions were finally raised. Nowadays, the ecological problem of sea dumped Chemical Warfare Agents is slowly getting its deserved recognition. Sea dumped CW munitions are being found worldwide, there is an observation of constant release of their fillings, both parent agents and their degradation products turn to be very toxic or potentially harmful what poses a serious threat in a perspective of their bioaccumulation in food webs. As a continuation of multiyear sea-dumped munition research following MERCW, CHEMSEA and MODUM projects, DAIMON project provides broad but balanced information on the problem. Series of observations from OECD toxicity tests performed in MUT laboratories and multiple field studies in the Baltic Sea area, supported by ECOPATH with ECOSIM modeling of the contaminants bioaccumulation in Baltic Sea food webs, are being put together to fill gaps in and between legislation, science and technology creating holistic ecological insight of the threats originating from sea dumped CWA. But first of all, our research is providing a categorization of threats, based on the acute and chronic toxicity tests.



## A2.5 High Resolution Mass Spectrometry in Identification of Novel Chemical Warfare Agent-related Phenylarsenic Chemicals

Hanna Niemikoski, Martin Söderström, Paula Vanninen

Finnish Institute for the Verification of the Chemical Convention (VERIFIN), Helsinki, Finland

In DAIMON project, new approaches for analysis of chemical warfare agent (CWA)-related chemicals has been applied. High resolution mass spectrometry (LC-MS/HRMS) was used for analysis of sediment samples from CWA dumpsites. Samples containing high concentrations of phenylarsenic CWAs collected in the CHEMSEA and DAIMON projects were selected and screened by LC-HRMS technique. Novel methylated phenylarsenic chemicals were detected from these samples. The elemental composition of these methylated phenylarsenic CWAs were confirmed using synthesized reference chemicals. Other new phenylarsenic chemicals were also detected and their structure elucidation is in progress.

The LC-MS/HRMS technique was also applied to confirm the presence of oxidised form of triphenyl arsine and Clark I/II in marine biota samples with concentrations below limits of detection. In in vitro metabolism studies (S9 fraction of cod liver homogenates and rainbow liver cell lines) with oxidised form of Clark I/II, novel metabolites has been identified using LC-MS/HRMS. None of these metabolites have ever been studied from marine biota samples and methods must be developed to enable further investigations on their possible health risks for marine biota and human consumers.

## A2.5 Detection of Chemical Warfare Agent-Related Phenylarsenic Chemicals From Different Marine Biota samples

Hanna Niemikoski, Tomi Rautanen, Paula Vanninen

Finnish Institute for the Verification of the Chemical Convention (VERIFIN), Helsinki, Finland

After World War II, disposal of chemical warfare agents (CWAs) began by sea-dumping. Hundreds of thousands of tons of chemical ammunitions (mainly sulfur mustard and phenyl arsenic compounds) were dumped in the Baltic Sea and Skagerrak area between Norway and Denmark. During previous international projects (MERCW (2005-09), CHEMSEA (2011-14) and MODUM (2013-16)) investigations have shown that sediment samples collected near dumping areas are contaminated with CWAs as a result of leaking containers. These leaking toxic chemicals are posing a threat to marine environment. In aqueous environment phenylarsenic chemicals degrade forming hydrolysis and oxidation products. There are hardly no previous studies of possible uptake of CWA-related chemicals by marine biota. During DAIMON project novel methods for detection of phenyl arsenic chemicals from muscle, gills and liver samples were developed. Quantitative analysis of these chemicals and their degradation products are needed to prove presence of these products in aquatic biota in order to support risk assessment for possible accumulation in food chain. Totally 187 marine biota samples (eg. cod, saithe, flatfish, lobster, shrimp, hagfish) were collected from three different dumping areas in the Baltic Sea and North Sea areas and analysed. Also 120 cod samples from collected from the Gdansk deep reference area were analysed for CWA-related phenylarsenic chemicals. The target matrix was fish muscle, but also some of the liver and gills samples were analysed. 25 % of analysed samples contained CWA-related phenylarsenic chemicals, diphenylarsinic acid (DPA[ox]) and triphenylarsine oxide (TPA[ox]). Concentrations of detected chemicals were at parts per billion (ppb, ng/g) concentration levels. This is the first time when CWA-related chemicals have been detected from marine biota species which are widely used as human nutrition. Arsenic-containing chemicals have been detected from marine biota samples as their pentavalent oxidation state. Due to the lack of information how these agents behave in living organism it is impossible to predict interactions or transformations of these chemicals. Based on that, more information is needed to elucidate total phenylarsenic concentrations in marine biota samples.

## A2.5 Toxicity of an oxidation product and a metabolite of the chemical warfare agent Clark I/II determined using the rainbow trout liver cell line RTL-W1

Hanna Niemikoski<sup>1</sup>, Kari Lehtonen<sup>2</sup>, Aino Ahvo<sup>2</sup>, Ilse Heiskanen<sup>3</sup>, Paula Vanninen<sup>1</sup>

<sup>1</sup> Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN), Helsinki, Finland

<sup>2</sup> Finnish Environment Institute, Marine Research Centre, Helsinki, Finland

<sup>3</sup> Finnish Environment Institute, Laboratory Centre, Helsinki, Finland

After the first and second world wars, thousands of tonnes of chemical warfare agents (CWAs) were dumped in the Baltic Sea. Recent studies have found CWA-related arsenical compounds accumulated in fish tissues. The potential effects of dumped chemical munitions in Baltic Sea marine biota are still poorly understood. To link the measured chemicals in the fish to biological effects, cytotoxicity of diphenylarsenic acid (DPA[ox]) an oxidation product of the chemical warfare agent Clark I/II, was studied using the rainbow trout liver cell line RTL-W1. Additionally, toxicity of diphenylarsine glutathione conjugate (DPA-SG), which is a metabolite of DPA[ox], was also studied. Cytotoxicity of the compounds was evaluated using the Neutral Red retention test (NRR), showing a LC<sub>50</sub> value of 294 mg/L for DPA[ox] and 1.32 mg/L for DPA-SG, indicating that the glutathione conjugate of DPA[ox] is more than two orders of magnitude toxic than the DPA[ox] itself. The kinetics and metabolism of DPA[ox] were also studied by incubating the RTL-W1 cells in media with 100 mg/L DPA[ox] and measuring the CWA-related phenylarsenic compound taken up by the cells by ultra-high performance liquid chromatography connected to high resolution mass spectrometry (UPHLC-HRMS). Also, DPA[ox] concentration in the cell media was measured in order to compare the fate of DPA[ox] in cell media to intracellular DPA[ox] concentrations after 30 min, 1 h, 2 h, 3 h and 4 h exposure.

Toxicity of trinitrotoluene (TNT), a conventional explosive found in many dumped conventional munitions, was studied with the same method, and the LC<sub>50</sub> for this substance was found to be 61 mg/L.

These studies will elucidate the biological effects of dumped conventional and chemical munitions, and help in assessing the environmental and health risks posed by their continued presence and deterioration in the sea bottoms.

## A2.5 In vitro effects of Trinitrotoluene on fish liver enzymes

Daniel Koske<sup>1</sup>, Nadine I. Goldenstein<sup>1</sup>, Timothy Rosenberger<sup>2</sup>, Ulrike Machulik<sup>1</sup> and Ulrike Kammann<sup>1</sup>

<sup>1</sup>Thünen Institute of Fisheries Ecology, Bremerhaven, Germany

<sup>2</sup>Carl von Ossietzky University Oldenburg, Institute for Chemistry and Biology of the Marine Environment (ICBM), Oldenburg, Germany

Trinitrotoluene (TNT) in dumped ammunition on the seafloor is subject to various biotic and abiotic transformation processes in the marine environment. Understanding these conversion reactions is important when evaluating TNT-induced effects on fish, and consequently on humans. Since its derivatives are known to cause different biological effects compared to TNT itself, this study addresses the potential effects of TNT on biotransformation enzymes and the associated formation of metabolites in fish.

Experiments to simulate TNT-metabolic processes in-vitro were conducted by exposure of liver S9 fractions from fish of the Baltic Sea to the toxicant. Activity of different hepatic enzymes was measured via emitted fluorescence while metabolites were analysed by HPLC-MS/MS.

In this study, consistent with previous in-vivo observations, we demonstrate that TNT affects the efficiency of important biotransformation enzymes in fish. The experiments revealed a time-dependent transformation of TNT to specific metabolites via liver-enzyme catalysed in-vitro conversions.

We provide valuable information on toxicity in fish caused by TNT, released from dumped ammunition. The analysis of metabolites, in addition to the TNT molecule, strongly improves our knowledge on environmental impacts of explosives. Hence, future studies and monitoring activities on fish from munition dumpsites should include the screening for TNT-metabolites.

## A2.5 Genotoxicity of nitroaromatic compounds in zebrafish embryos

Daniel Koske and Ulrike Kammann

Thünen Institute of Fisheries Ecology, Bremerhaven, Germany

Environmental contamination of the seafloor with Trinitrotoluene (TNT) is a well-known problem. Since TNT is subject to different degradation processes, marine organisms such as fish are getting in contact with a broad variety of nitroaromatic explosive contaminants. Besides its acute toxicity TNT is known for its mutagenic effect on bacteria strains. Also, the urine of TNT exposed rats showed mutagenicity in former studies.

Our study is the first one that uses a specific in vivo approach to address genotoxicity of TNT and two of its major metabolites, 2-amino-dinitrotoluene (2-ADNT) and 4-amino-dinitrotoluene (4-ADNT), in zebrafish embryos. Investigating the potential genotoxicity of TNT in zebrafish embryos offers a detailed and reliable model for assessing the potential risk for fish residing close to munition dumpsites.

Zebrafish embryos were exposed over 48 hours to sublethal concentrations of TNT, 2 ADNT and 4 ADNT. Genotoxicity in zebrafish embryos was investigated by following the principles of the alkaline comet assay. DNA damage in the individual cells was compared by using the olive tail moment.

Here we demonstrate novel evidence that TNT, as well as the major degradation products 2 ADNT and 4 ADNT, have a significant genotoxic effect in zebrafish embryos. After 48 hours even the lowest tested concentration of degradation products (1 mg/l) led to a remarkable increase of genotoxicity compared to the control.

These results contribute to a better understanding of explosive related effects in fish. Furthermore, genotoxic effects in fish caused by TNT and its degradation products can serve as explanation for long-term effects such as tumors in fish from munition dumpsites.

## A2.5 Fluorescence measurements of the marine flatworm *Macrostomum lignano* during exposure to the TNT derivatives 2ADNT and 4ADNT

Ulf Bickmeyer, Ina Meinen, Stefanie Meyer, Matthias Brenner

Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Bremerhaven, Germany

The most significant explosive of conventional munition dumped in the sea is 2,4,6-trinitrotoluene (TNT). Two main metabolites of TNT are 2-amino-4,6-dinitrotoluene (2ADNT) and 4-amino-2,6-dinitrotoluene (4ADNT). The toxicity of both compounds was tested on the marine flatworm *Macrostomum lignano* raised in the laboratory. The tested concentrations of both derivatives were in the  $\mu\text{M}$  to the  $\text{nM}$  range. In addition, the influence of temperature and UV-radiation was determined. By increasing the temperature to  $30^\circ\text{C}$ , the toxicity of both derivatives was strongly increased.

Using sub-lethal concentrations detoxifying defense mechanisms of exposed worms, namely drug and xenobiotic transporters, expelling toxic compounds from the cytosol were strongly enhanced. Also measurements of auto-fluorescence of exposed flatworms showed distinct alterations.

## A2.6 Risk assessment model for dumped Chemical Warfare Agents – VRAKA-CWA

Andreas Lindhe<sup>1</sup>, J. Fredrik Lindgren<sup>2</sup>, Lars Rosén<sup>1</sup>, Anders Tengberg<sup>2</sup>, Ida.-Maja Hassellöv<sup>2</sup>

<sup>1</sup>Chalmers University of Technology, Department of Architecture and Civil Engineering, Gothenburg, Sweden

<sup>2</sup>Chalmers University of Technology, Department of Mechanics and Maritime Sciences, Gothenburg, Sweden

Succeeding the end of WWII, large amounts of conventional and chemical munitions were dumped in deep water areas in the Baltic Sea and Skagerrak. Approximately 65,000 ton of chemical warfare agents (CWA), e.g. Yperite (mustard gas), Clark I, Clark II, Lewisite and Adamsite, were dumped between 1947–1948. To enable structured assessments of the risk associated with dumped CWAs, a new and novel model (VRAKA-CWA) has been developed and applied. The model is used to calculate the probability of different activities causing damage to a munition unit and thus having a release of CWA. The risk is calculated as a function of the probability, the toxic unit of the specific CWA, and the mass of CWA. The results, e.g. the probability of release and the final risk level, is illustrated as cumulative distribution functions, representing uncertainties in input data and results. When using the model, the assessor defines eight site-specific indicators such as salinity and depth. Furthermore, an estimation is done of the number of times per year each of seven different types of activities, such as diving and trawling, occurs at the specific site and may cause damage to the unit being assessed. Finally, the arrangement of the munition units and type of CWA they contain are defined. In the model, a Bayesian updating is performed to combine data on generic conditions with the site-specific data to obtain probability and risk estimates for each CWA unit being analysed. Unit-specific risk estimations can be integrated to area specific risk estimations. VRAKA-CWA makes it possible to compare, in a structured way, the risk level associated with different CWA units and areas. Furthermore, it is possible to compare to what extent different activities (e.g. diving and trawling) and natural processes (corrosion and unstable seabed/landslides) contribute to the overall probability and thus the overall risk. Hence, VRAKA-CWA provides important decision support that has previously been lacking.



### A3.1 Blue mussels (*Mytilus* spp.) transplanted at a German munition dumping site in the Baltic Sea for biomonitoring of TNT and degradation products

Edmund Maser, Daniel Appel, Hans-Jörg Martin, Jennifer S. Strehse

Institute of Toxicology and Pharmacology for Natural Scientists, University Medical School, Kiel, Germany

Since World War I considerable amounts of warfare materials have been dumped at sea worldwide, but little is known about the fate of the explosive components in the marine environment. Sea dumped munitions are able to contaminate the surroundings because of the release of explosive chemicals due to corrosion and breaching or by detonation after blast-operations. This implies the risk of accumulation of toxic compounds in human and wildlife food chains.

With the help of divers, we performed an active biomonitoring study with transplanted blue mussels (*Mytilus* spp.) in a burdened area (Kolberger Heide, Germany) with explosive compounds near blast craters. Furthermore, we transplanted mussels in vicinity to a mine mound with over 100 moored mines. In order to monitor any differences resulting from changing seasons, three exposure periods for mussels exposed at the mine mound were chosen.

In all mussels deployed at the ground next to the bulk of hexanite, we found a body burden with 2-amino-4,6-dinitrotoluene (2-ADNT) of  $103.75 \pm 12.77$  ng/g wet weight and with 4-amino-2,6-dinitrotoluene (4-ADNT) of  $131.31 \pm 9.53$  ng/g wet weight. 2,4,6-trinitrotoluene (TNT) itself has been found in six mussels with an average concentration of  $31.04 \pm 3.26$  ng/g mussel wet weight. In the mussels positioned at one meter above the ground no TNT nor 2-ADNT could be detected, but 4-ADNT was found in all samples with an average concentration of  $8.71 \pm 2.88$  ng/g mussel wet weight.

In mussel tissues from the first exposure period (April – July 2016, 106 days) 4-ADNT ranging from  $3.37 \pm 1.01$  to  $7.76 \pm 6.53$  ng/(g mussel wet weight) could be detected in all samples at each mooring position. After the second exposure period (July – December 2016, 146 days) 4-ADNT ranging from  $3.04 \pm 1.30$  to  $4.94 \pm 1.60$  ng/(g mussel wet weight) has been found in 34 out of 36 samples. After the third exposure period (December 2016 – March 2017, 92 days) 4-ADNT levels ranging from  $4.26 \pm 1.40$  to  $7.01 \pm 2.41$  ng/(g mussel wet weight) were detected in mussels from recovered positions. Neither TNT nor 2-ADNT have been found in any sample.

With this biomonitoring system, we could show that blue mussels accumulate TNT and its metabolites 2-ADNT and 4-ADNT in their tissues and we unequivocally proved that toxic explosives accumulate in the marine biota resp. in the marine food chain, thereby posing a possible risk to the marine ecosphere and human health.



### A3.1 Biological effects in mussels (*Mytilus* sp.) growing on depth charges in the Gulf of Finland

Aino Ahvo<sup>1</sup>, Anu Lastumäki<sup>1</sup>, Raisa Turja<sup>1</sup>, Jennifer Strehse<sup>2</sup>, Edmund Maser<sup>2</sup>, Kari Lehtonen<sup>1</sup>

<sup>1</sup>Finnish Environment Institute, Marine Research Centre (SYKE), Helsinki, Finland

<sup>2</sup>Institute of Toxicology and Pharmacology for Natural Scientists, University Medical School, Kiel, Germany

After the world wars, hundreds of tons of conventional munitions (mines, charges and shots) were dumped into the Baltic Sea. After 70 years in the sea, the fate of the corroded munitions and explosive chemicals in the marine environment is unknown. In the present study the possible biological effects of deteriorated conventional munitions were evaluated in the Baltic Sea blue mussels (*Mytilus* sp.) living on depth charges on a WW2 wreck in the Gulf of Finland.

Mussels were collected on top of intact depth charges as well as one broken depth charge, and from a reference area nearby. Biomarkers of neurotoxicity, oxidative stress damage, antioxidant enzyme system, lysosomal integrity and lipofuscin accumulation were analyzed in mussel tissues. Whole tissue samples were also analyzed for traces of the explosive trinitrotoluene (TNT) and its degradation products, as well as polycyclic aromatic hydrocarbons (PAHs) and selected metals to observe the presence of hazardous chemicals related to deteriorated depth charges or contaminated sediments in the area.

Mussels sampled from the depth charges showed significant differences in acetylcholinesterase, glutathione reductase and catalase activity as well as lysosomal membrane stability and lipid peroxidation compared to the reference mussels. The results of the biomarker analyses show that mussels sampled close to depth charges seem to be experiencing some oxidative stress. However, no TNT was found in any of the whole mussel chemical samples. Other contaminants, such as metals, may account for the elevated stress levels seen in the mussels living on top of the depth charges.

### A3.1 Studies on the health status of dab (*Limanda limanda*) from a dumpsite of conventional munitions in Kiel Bight, Baltic Sea

Katharina Straumer and Thomas Lang

Thünen Institute of Fisheries Ecology (TI-FI), Bremerhaven, Germany

The impact and effects of dumped conventional munitions on fish health in the Baltic Sea has been studied with a focus on dab (*Limanda limanda*). Dab is the most abundant flatfish species in the area, and has been used as a marine bioindicator species in many studies on biological effects of contaminants carried out in the Baltic Sea and North Sea.

In the present study, dab was examined for fitness indicators and the presence of externally visible diseases and parasites and a special emphasis on macroscopic and histopathological liver lesions, including tumours. Methodologies applied followed standardized ICES and BEQUALM protocols. Sampling areas were the munitions dumping area Kolberger Heide (Kiel Bight), compared to Flensburg Fjord, Little Belt and two reference areas in Kiel Bight considered free of dumped munitions, located north and west of Kolberger Heide.

In total, the health status of 1143 dab was analysed. There was no significant difference in condition factor (used as fitness index) between the study areas. Also the examination for externally visible diseases and parasites did not reveal significant differences between the areas investigated. However, the results of the examination of dab livers for macroscopic and microscopic lesions indicate a significantly higher prevalence of neoplastic liver lesions (tumours and pre-stages) in fish from the dumpsite Kolberger Heide. In addition, a specific and rare liver disease (peliosis hepatis) was recorded in dab from the dumping area. The results are discussed in relation to available data on the presence of dumped munitions and the release of compounds in the study areas as well as on known toxic effects of contaminants associated with dumped conventional munitions.

### A3.1 Dumped munitions in the Baltic Sea – Detection and effects of explosives in fish

Nadine I. Goldenstein, Daniel Koske, Katharina Straumer, Thomas Lang and Ulrike Kammann  
Thünen Institute of Fisheries Ecology (TI-FI), Bremerhaven, Germany

The seafloor of the Baltic Sea holds more than 200 000 tons conventional munitions originating from the World War I and II. These explosives are mostly based on nitroaromatic compounds of which Trinitrotoluene (TNT) represents the most common substance. TNT is subject to a variety of biotic and abiotic transformation processes in the marine environment. These degradation reactions include denitration as well as reduction of nitro to nitroso, hydroxylamine and finally amino derivatives. These products are expected to cause biological effects distinct from their precursor due to their distinct oxidation state, which is known to affect the intensity and mode of protein interaction.

Hence, it is of utmost importance to understand the effects of munitions compounds like TNT and its metabolites on fish as a marine organism residing in close proximity to submarine dumpsites and representing one of the main food sources for our modern society, especially since progressive deterioration of the explosive devices provides a long-term source for exposition to these xenobiotic substances. The work conducted at the TI-FI within the DAIMON project identified relevant metabolites of TNT in vitro by using S9-liver fractions. Furthermore we have proven the genotoxic potential of these degradation products in relation to their precursor in acute exposition tests with zebrafish embryos. The acquired knowledge on metabolism was applied to track TNT and its derivatives in wild-caught fish (common dab, *Limanda limanda*) collected in the dumpsite Kolberger Heide, Kiel Bight, as well as in fish from reference sites. Fish from the dumpsite showed bioaccumulation of explosives-related compounds and, at the same time, an increased prevalence of neoplastic and other toxicopathic lesions in the liver, the most relevant organ for detoxification. It can, thus, not be excluded that TNT and its metabolites cause genotoxic/carcinogenic effects in wild fish from the dumpsite.

Here, for the first time bioactivity of nitroaromatic explosives in fish have been demonstrated in vitro as well as in vivo. These results provide a sound basis for a detailed assessment of consequences of dumped munitions for wild fish. Furthermore, the knowledge gained on relevant target substances and biological effects enables a long-term monitoring and risk evaluation related to ecosystem effects of dumped conventional munitions.

### A3.2 Using the hagfish (*Myxine glutinosa*) to study biological effects of a wreck filled with chemical munitions

Aino Ahvo<sup>1</sup>, Hanna Niemikoski<sup>2</sup>, Katharina Straumer<sup>3</sup>, John Aasulf Tørnes<sup>4</sup>, Paula Vanninen<sup>2</sup>, Thomas Lang<sup>3</sup>, Anu Lastumäki<sup>1</sup>, Kari Lehtonen<sup>1</sup>

<sup>1</sup>Finnish Environment Institute, Marine Research Centre, Helsinki, Finland

<sup>2</sup>Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN), Helsinki, Finland

<sup>3</sup>Thünen Institute of Fisheries Ecology (TI-FI), Bremerhaven, Germany

<sup>4</sup>Norwegian Defense Research Establishment (FFI), Kjeller, Norway

The sea bottom of the Skagerrak Strait (North Sea) contains ca. 45,000 tonnes of chemical warfare agents (CWA) dumped after the Second World War. Entire ships loaded with of CWA were intentionally sunk and are still laying on the deep bottoms (ca. 600 m) of the area in different states of deterioration by corrosion. The current status of the CWA in the wrecks is unknown; if released into the environment they may have significant deleterious effects on local marine biota. Within the research programme of the EU Baltic Sea Region Interreg project DAIMON (Decision Aid for Marine Munitions, [www.daimonproject.com](http://www.daimonproject.com)), one of these wrecks was selected to study the leakage of CWA and their possible biological effects. From the few fish species that inhabit the studied depth range in the region, the hagfish (*Myxine glutinosa*), a sediment-dwelling chordate, was selected as target organism for chemical analyses of CWA in tissues and biological effect studies. Samples were taken using bait traps near the wreck and from a reference area known to contain no wrecks. Liver tissue was analysed for oxidative stress biomarkers (including lipid peroxidation, glutathione-S-transferase, glutathione reductase and catalase activity) and for histopathological biomarkers, and muscle tissue was analyzed for acetylcholinesterase activity. Chemical analyses were performed from muscle samples and separate whole fish samples, and the results indicated the presence of oxidized forms of CWA-related phenylarsenic compounds in most of the muscle samples. Established biomarker methods used widely in various fish species were shown here for the first time to be applicable also in hagfish. However, only minor differences in the measured biomarker responses between individuals collected from the wreck and the reference area could be observed. Based on this study, the hagfish is regarded as a suitable candidate for ecotoxicological studies of deep marine areas. More information on the biology of hagfish and the natural variability of their biomarkers is needed to distinguish true effects of hazardous substances.

### A3.2 Case studies on wrecks filled with chemical munitions

John Aa Tørnes

Norwegian Defence Research Establishment(FFI), Kjeller, Norway

Two DAIMON cruises were conducted in June 2017 and in July 2018 to the dumping area for ships filled with chemical munition south-east of Arendal in Norwegian waters. In addition, one test cruise in the same area was conducted in January 2018 to evaluate new sampling equipment. During these three cruises, biota samples (mostly hagfish) were collected at positions close to Wreck no 13 and at distances 1.5 km, 2.2 km and 8 km from the wreck. Before the DAIMON project, sediment samples have been collected in 2002 and 2015 close to this wreck. During DAIMON, one multi-incremental sample, where all slices from the same depth from all cores taken in the same square were mixed to one common sample (i.e. one common sample for each depth). Such mixed samples are better suited than spot samples for taking decisions on possible remedial actions in areas with very inhomogeneous concentrations of chemical compounds in the sediment.

### A3.3 Results of case studies on dumped chemical munition in the area of Bornholm Deep and Gdansk Deep conducted during DAIMON project (2016 – 2019).

Miłosz Grabowski

Institute of Oceanology of the Polish Academy of Sciences (IOPAN), Sopot, Poland

During the duration of the DAIMON project (2016-2019) several scientific expeditions were conducted by the IO PAS team in the areas of chemical munition dumpsites on the Baltic Sea – Bornholm Deep and Gdansk Deep. In this presentation, the methodology of surveying of underwater dumpsites will be widely presented. Also, the results of acoustic surveys with the usage of AUV will be revealed, as well as outcomes of photographic and photometrical investigation performed by the ROV on detected objects will be discussed. Collected data provided detail information, which was crucial to conduct objects identification and risk assessment in study areas. Additionally, examples of detected objects will be presented and procedure of deploying and recovery of passive samplers near confirmed objects will be shown.

### A3.3 Overview of the results of the monitoring campaigns for analysis of Baltic Sea sediment samples for sea-dumped chemical warfare agents

Paula Vanninen<sup>1</sup>, Hanna Lignell<sup>1</sup>, Stanisław Popiel<sup>2</sup>, Jacob Nawala<sup>2</sup>, Galina Garnaga<sup>3</sup>, Pączek Bartłomiej<sup>4</sup>, John Tørnes<sup>5</sup>, Marta Szubska<sup>6</sup>, Jacek Bełdowski<sup>6</sup>

<sup>1</sup>Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN), Helsinki, Finland

<sup>2</sup>Military University of Technology (MUT), Gdansk, Poland

<sup>3</sup>Environmental Protection Agency, Vilnius, Lithuania

<sup>4</sup>Polish Naval Academy, Gdynia, Poland

<sup>5</sup>Norwegian Defence Research Establishment (FFI), Kjeller, Norway

<sup>6</sup>Institute of Oceanology of the Polish Academy of Sciences (IOPAN), Sopot, Poland

After the Second World War, large amounts of chemical munitions containing chemical warfare agents (CWAs) were dumped into the Baltic Sea. In 2006-2018, Bornholm, Gotland deep, Skagerrak, and Gdansk deep dumpsites were studied in many projects firstly in the EU FP6 project Modelling of Ecological Risks Related to Sea-Dumped Chemical Weapons (MERCW). This project was later amended by Baltic Sea Region Interreg Project Chemical Munitions, Search and Assessment (CHEMSEA), NATO SPS project Towards the Monitoring of Dumped Munition Threats (MODUM) and a joint EU project Decision Aid for Marine Munitions (DAIMON). The presence of various CWAs was studied. The results indicate a widespread contamination reaching far beyond the dumpsite boundaries, as CWA degradation products were detected in most of the sediment samples taken from and outside the dumpsites. The contamination is mostly related to arsenic containing compounds like Clark I/II, Adamsite, arsenic oil, and inorganic arsenic. Some samples also indicated the presence of sulfur mustard and its degradation products. Due to the presence of multiple, overlying sources of contaminants, the correlation between detected compounds and CWA concentrations is not always straightforward. However, overall results strongly suggest that the sea-dumped munitions are indeed leaking and the contamination has eventually reached the seafloor sediments. Overview of the analysis results and located hotspots are visualized in the geographical maps. The analysis data is also fully loaded into a Decision Support System (DSS), which produced in the DAIMON project is available for authorities, providing risk assessment and decision aid related to operations in the contaminated areas.

### A3.3 Integrated biomarker analysis and assessment in cod (*Gadus morhua*) from dumpsites of chemical munitions in the Baltic Sea

Katharina Straumer<sup>1</sup>, Kari K. Lehtonen<sup>2</sup>, Anu Lastumäki<sup>2</sup>, Raisa Turja<sup>2</sup>, Aino Ahvo<sup>2</sup>, Matthias Brenner<sup>3</sup>, Thomas Lang<sup>1</sup>

<sup>1</sup>Thünen Institute of Fisheries Ecology (TI-FI), Bremerhaven, Germany

<sup>2</sup>Finnish Environment Institute, Marine Research Centre, Helsinki, Finland

<sup>3</sup>Alfred Wegener Institute of Polar and Marine Research (AWI), Bremerhaven, Germany

Dumping of chemical munitions and chemical warfare agents (CWA), mainly from German production, took place in the deep basins of the Baltic Sea after WW 2 by order of the allied forces, and it is estimated that between 30,000 and 50,000 t were disposed. In the framework of the CHEMSEA project (2011-2013), cod (*Gadus morhua*) from CWA dumpsites located in the Bornholm Basin, Gotland Deep and Gdansk Deep as well as from a munitions-free reference site outside the Gdansk Bay were studied for spatial patterns in biomarker responses. A battery of established biomarkers was analysed, reflecting exposure, early or chronic contaminant effects. These included enzymatic (acetylcholinesterase, glutathione S-transferase, glutathione reductase, catalase), cell and tissue (lysosomal membrane stability, lipofuscin accumulation, apoptosis, liver histopathology) as well as fitness and health biomarkers (condition index, diseases and parasites). For the integrated analysis of biomarker data obtained, a modified version of the Integrated Biomarker Response (IBR) approach was applied, introducing weighting factors for each biomarker as an additional component, emphasizing the significance of the response/effect for the host. The results reveal marked spatial differences in the IBR results, with the major CWA dumpsite (Bornholm Basin) showing the strongest and the reference site outside Gdansk Bay the lowest multi-biomarker response. For the assessment, also an integrated approach is suggested that either utilizes established or newly developed assessment criteria (BAC: background assessment criteria; EAC: environmental assessment criteria) for each biomarker, which are combined into an integrated multi-biomarker assessment based on individuals and sampling areas.



### A3.3 Development of techniques for monitoring of the dynamics and structure of water on the route of relocatable bottom sediments

Vadim Paka

Russian Academy of Sciences, Shirshov Institute of Oceanology, Kaliningrad, Russia

The main quantity of the sea-dumped chemical weapons (CW) in the Baltic Sea is located in the central part of the Bornholm Deep in the area with soft pelitic sediments capable to absorb polluting substances and characterized by low threshold of resuspension velocity. This area is located on a pathway of inflowing salt dense waters, so, the impact of the environment on the CW, the release of toxic substances from their containers and their relocation vary widely. Toxic materials together with light weighted particles are transported by inflow currents far beyond the dumpsite and can be discharged in certain parts of the route, again washed away and transferred to a new location.

The conclusion is that there is a need for permanent monitoring of the state and movement of water, especially in the bottom boundary layer. Common monitoring methods do not have sufficient information about the fine structure and dynamics of the thin bottom layer. Our efforts were aimed at eliminating this shortcoming. The report proposes two proven solutions to this problem: 1) obtaining a detailed structure of the waters at all depths by means of vertical profiling from the moving ship, 2) measurement of bottom currents in a large number of points on the system of transects or polygons with the use of moored tilt current meters.

To carry out these measurements, there is no need for specially equipped vessels, which radically expands the possibility of further development of monitoring of changing conditions of chemical contamination stay in the Baltic Sea.

#### A4.1 Economic and social analyses in the chemical and conventional warfare dumping areas of the Baltic Sea - cost-benefit analysis

Lech Kotwicki and Michał Czub

Institute of Oceanology of the Polish Academy of Sciences (IOPAN), Sopot, Poland

Ecosystem services are defined as the functions and processes through which ecosystems, and the species that they support, sustain and fulfil human life and can be categorised in different ways. For ecosystem services classification, the approach from HELCOM (2010) and the Millennium Ecosystem Assessment Report (Millennium Ecosystem Assessment, 2005) was applied. Relative importance of each respective ecosystem service for Skagerrak, Bornholm Basin and Kolberger Heide were selected, regarding the economic benefits of ecosystem services, the cost of measures required to protect these services, as well as the estimated costs of non-action.

#### A4.2 IDUM – evaluation the costs of different management options (e.g. sediment covering, remediation, etc.), and list of possible environmental impacts according to available technology used

Cherylynn Hunt

Internal Dialog on Underwater Munition (IDUM), The Hague, Netherlands

Management strategies are effective means to deal with many dump sites. Realistically, the entire problem cannot be solved without fiscal strategic employment of site-specific remedies. A higher risk site will always take priority of a lessor one; yet, all need to have a management strategy for holistic eventual clean up. It is complicated to give an exact calculation on the costs of the different management options; however, a general evaluation can be made to illustrate the many aspects required of specific operations with their annual costs. Essentially, six factors must be considered in evaluating the costs of: No Action, Monitoring, Limiting Certain Actions at Sea, Neutralizations at Sea, Detonation in Situ and Recovery and Destruction operations such as: size of the site, duration of the visits, specific activity that needs done, equipment needed, personnel involved, and consumables needed. Based on case studies from the past, assumptions can be made for evaluations of (future) operations and the resources required. Technological advancements have cleared the way for safe, environmentally friendly, and cost-effective remediation of many of today's sites; however, some sites due to the complexities of access, environmental factors and risk may not be presently likely candidates for remediation due to high costs. Most importantly, detailed planning through updated historical reviews, surveying, site sampling, risk identification and mitigation considerations are necessary prior to any remediation efforts.

A4.3 IDUM – legal situation, how are dumped items/sites and responsibilities regulated, what are the consequences for dump site in national/international waters due to existing legal framework, what is missing in the legal framework, etc.

Cherylynn Hunt

Internal Dialog on Underwater Munition (IDUM), The Hague, Netherlands

The issue of underwater munitions is still facing the world today. The recovery of underwater munitions has a lot of obstacles, but actions are required to be taken on national and international levels. The research of legal aspects of underwater munitions management identifies that the laws on underwater munitions management are fragmented and uneven and that there is no consistency in legislation, nor any common language, regulations, or mandates.

IDUM has researched different levels of Law in relation to the underwater munitions' management around the world, starting from the national aspects further going to the international perspective. With no common existing treaty, protocol, or Convention, overall approach to the Law of the Sea is not harmonized and is fragmented at best. No current domestic and international laws cover the underwater munitions common approach.

Currently, there are many treaties that are relevant to the issue of underwater munitions, but there is no joint one. Existing Treaties that are relevant to the issue of underwater munitions include Chemical Weapons Convention (CWC), 1958 Convention on the High Seas and 1974 Convention on the Protection of the Marine Environment of the Baltic Sea Area. In addition, the CWC has another approach of handling sea-dumped weapons. The "1985 exemption" of CWC states all marine munitions prior to 1 January 1985 are not covered by the Convention.

#### A4.4 The Måseskär dump site – concentrations of chemical warfare agents, and possible management options

J. Fredrik Lindgren<sup>1</sup>, Ingela Dahllof<sup>2</sup>, Vadim Paka<sup>3</sup>, Hanna Niemikoski<sup>4</sup>, Lech Kotwicki<sup>5</sup>, Anders Tengberg<sup>1</sup>, Paula Vanninen<sup>4</sup>, Ida-Maja Hassellöv<sup>1</sup>

<sup>1</sup>Chalmers University of Technology, Mechanics and maritime sciences, Gothenburg, Sweden

<sup>2</sup>University of Gothenburg, Biological and Environmental Sciences, Gothenburg, Sweden

<sup>3</sup>Russian Academy of Sciences Shirshov, Institute of Oceanology, Kaliningrad, Russia

<sup>4</sup>Finnish Institute for the Verification of the Chemical Convention (VERIFIN), Helsinki, Finland

<sup>5</sup>Institute of Oceanology of the Polish Academy of Sciences (IOPAN), Sopot, Poland

##### 1. Introduction

Succeeding the end of WWII, there were large stockpiles of conventional and chemical munitions in the northern parts of former Nazi Germany. This constituted a large problem and it was decided that damaged or old ships were to be filled with munitions from the stockpiles and sunk in deep water areas in the Baltic Sea and Skagerrak. Approximately 65 000 ton chemical warfare agents (CWA), e.g. Yperite (mustard gas), Clark I, Clark II, Lewisite and Adamsite, were dumped in the sea between 1947-1948. About 50 % was dumped in the Baltic Sea, mostly in the Bornholm basin [1]. The dumped material was in the form of shells, grenades, mines, bombs but also wooden encasements, containers and small drums [2]. Another deep-water area dumpsite was in the Skagerrak area, Maseskar (27x 21 km, 259756, 6450499 [SWEREF 99]), where unconfirmed information exists of if and how much CWAs were dumped after WWII. The area is a known good spot for commercial fishing and trawling is performed daily. Furthermore, the area has been investigated during a few expeditions over the years. In a more recent survey 2015 the area was also thoroughly investigated in a hydrographic survey using side scan sonar and multibeam, where 28 wrecks were detected. In addition, 13 of the wrecks were further investigated using Remotely Operated Vehicle (ROV) [3]. In 1992 low concentrations, 3.2-1.2 µg/kg, of Yperite (mustard gas) were detected in sediment in the vicinity of the wrecks [4]. In addition, during 2017-2018 low concentrations, 29-4.7 ng/kg of Clark I were found in Norway lobster (*Nephrops norvegicus*), Witch flounder (*Glyptocephalus cynoglossus*) and shrimp (*Pandalus borealis*) in the area [5]. During the DAIMON project, Chalmers with Shirshov institute of Oceanology performed a sediment sampling in the area. The results from prior and the recent study were then used as base to investigate and discuss possible management strategies for the dumping area.

##### 2. Materials and methods

An expedition to the Maseskar dumpsite area was performed between 17-22 of June 2017. It is located approximately 14 nm west of the island of Maseskar, on the Swedish west coast. The depth in the area is between 190-220 m, compared to the average depth in the adjacent areas, which is about 70 m. The water current from the sea floor and 25 meters up in the water column is mainly north westerly [3]. Five areas were sampled for sediment using a Niemisto corer (Ø 7cm). Two of the areas were in the vicinity of the ship wrecks (0.5-5 km), one site in between ship wrecks areas, one area north west of the area with the highest concentrations of wrecks (downstream, 24-29 km to the closest wreck) and the last area was a control area south of Maseskar 22-26 km from the closest ship wreck). At each area five sites were sampled, except for the area with highest concentration of wrecks (cluster 4), where 9 sites were sampled. At each site five cores were taken,

sliced at 0-3, 4-6, 7-10 and 11-15 cm. The sliced sediment from the five cores was then thoroughly homogenized and pooled and sediment was divided for analysis of CWA, As and meiofaunal community composition.

## 2.1. Results and discussion

Mustard gas or degradation products of mustard gas were detected in 15 (52%) of the sediment samples. Concentrations in those samples ranged from 3.42-0.10 µg/kg (dw), where the highest concentrations were found at the area C4. Average As concentrations differed between 21.6 - 6.7 µg/kg (dw) in the top seven centimetres. Average As concentrations from 0-3 cm depth differed significantly between the sampled areas ( $p < 0.001$ ). Meiofaunal community composition differed significantly ( $p < 0.01$ ) between the sampled areas. Arsenic was most responsible for this difference in meiofaunal community composition, of the tested environmental variables.

Several investigations in the Maseskar area show that CWA leaks into the sediment and can also be found in biota. Management strategies should be applied to limit the environmental effects and spreading of the CWA to surrounding areas. Firstly, commercial fishing should be limited, as there is an intense trawling fishery in the area. To avoid spreading of CWA by the trawl and trawlboards and to limit exposure of CWA to fishermen. However, bilateral agreements between countries regulating fishing areas and quotas prevents Sweden alone from introducing such regulations. Secondly, a cost analysis should be performed to investigate the possible costs for raising the dumped munitions and sending them for destruction. The alternative is to let the CWA stay on the seafloor and establish a monitor program, ensuring that the environmental effects isn't increasing. A third action that can be considered is containment of the CWA in the wrecks, by for example coverage with sediment masses or concrete. In addition, further studies in the area should be performed, to establish which wrecks contain and leaks CWA, and to establish the size of the area that should be closed for fishing and monitored.

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## A5.1 Specialist munitions software and database

Dietrich Steinmetz<sup>1</sup>, Sven Hartmann<sup>1</sup>, Klaus Koehler<sup>2</sup>

<sup>1</sup>University of Clausthal, Department of Informatics, Clausthal, Germany

<sup>2</sup>Dr. Koehler GmbH, Munster, Germany

The Baltic Sea contains 50000 tons of dumped chemical warfare agents (CWA) and more than 200000 tons of conventional munitions originating from the World Wars I and II. The Clausthal University of Technology has created a novel munition system in collaboration with Dr. Koehler GmbH, which allows to insert and manage complex munition types intuitively.

With the variety of the different dumped munition, the focus was on chemical munitions and the relevant dumped munition types. The detailed information about the munition includes parameters as length and diameter, case thickness, material, type of fuse and explosive, weight, chemical agent (such as Sulphur Mustard, Clark, Tabun, Phosgene). The parameters are important for the assessment of the risk, for the estimation of corrosion as well as the identification of the munition. Not only the munition itself but also some of the substances used in the munition pose a risk to the environment, to the food chain and to humans who are exposed to them. The main basis for the entered data was the research of internal archives with ammunition data from different sources.

The munition system is based on the open-source PHP web framework Laravel, which enables flexible development and maintenance. This allows existing munition types to be easily expanded or newly developed. Munition experts can use the web user interface to input, retrieve or update the munition data in a convenient way. The system provides a munitions database, which stores detailed information of dumped munition in a secure environment. The corresponding relational data model gives the possibility to process the data efficiently. Relevant munition data can be searched by different munition parameters. The munition data can easily be consumed via a REST-API and integrated into other systems such as DAIMON Decision Support System for marine munitions (DSS)- and may also be used for identification or as a knowledge base.



## A5.2 Innovative Neural Network Technology for DAIMON Decision Support System (DSS)

Sabine Bohlmann and Matthias Reuter

University of Clausthal (TUC), Department of Informatics, Clausthal, Germany

Based on the estimated 50.000 tons of dumped chemical warfare agent (CWA) and more than 200.000 tons of conventional munition from the world wars I and II, there is a high risk that during this century toxic contents are released into the Baltic Sea. In case of an ammunition finding the Baltic Sea governments and companies have to make a case-to-case decision depending on the kind and status of the ammunition, the actual environmental conditions and the protection goods in the surrounding (e.g. humans, flora and fauna, infrastructure). To assess the process a decision support system based on innovative neural network technology was developed within the DAIMON project. The core of the innovative approach is that all relevant and highly heterogeneous information for decision-making is processed and stored in a similar way like the brain of vertebrates. As our research has shown, this organisation principle of the brain - called "Computing with Activities" - is unique in order to handle the heterogeneity of the data with its wide ranges and situation relevant weighting and is in addition, robust against natural, environmental fluctuations.



### A5.3 Integration of DAIMON in AMUCAD: the actual status

Jann Wendt

EGEOS, Kiel, Germany

Based on the already existing visualization techniques for complex spatial data related to ammunition AMUCAD was chosen to provide the visual access to DAIMON via its interface. AMUCAD (the Ammunition Cadastre Sea) itself is a project developed by EGEOS GmbH which deals with the acquisition, management and analysis of ammunition related datasets for the North and Baltic Sea. Therefore, large amounts of historical and modern datasets are acquired and integrated into the system and new technologies are used for analysing and connecting these datasets. For the integration of DAIMON multiple steps had to be carried out including access to project related datasets and implementation of developed approaches:

- definition of functionality for interacting with DAIMON from inside of AMUCAD
- visual design of a user-friendly and user-experience centered interface including specialised requirements
- programming of planned functionalities and user interface
- testing and user rounds for incorporation of direct feedback
- development of an interactive online tutorial to provide an easy introduction into the functionality and interface of DAIMON

The actual status of the implementation provides already more than 90% of all measured datasets and planned functionalities of the final state. It provides an excellent starting point for the planned extension of DAIMON to include new useful functionalities and transform the prototype into a long lasting and user-friendly application.