WP3. Summary of chemical analysis of sediment samples

VERSION 1.0

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Attachment 3: Jenny Rattfelt Nyholm, Yvonne Nygren, Johanna Qvarnström and Anders Östin, *Analysis of chemical warfare related compounds in sediment samples from the Baltic Sea performed at the Swedish Defence Research Institute in the CHEMSEA project*.

Attachment 4: Stanisław Popiel, Jakub Nawała, Daniel Dziedzic, Joanna Błażejczyk, *Chemical analysis of sediment and core samples performed at Military University of Technology (MUT)*.

Attachment 5: Martin Söderström, Annette Pettersson, Maaret Karjalainen, Olli Kostiainen, Ullastiina Hakala, Terhi Taure, Matti Kuula and Paula Vanninen, *Chemical analysis of sediment samples performed at Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN)*.

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1. Introduction

This summary report is based on reports of the participants of the Work Package 3 of the CHEMSEA project. The original reports can be found in Attachments 3–6 of this report.

1.1 Background

After the second world war about 50 000 tonnes of chemical munitions were dumped in the Baltic Sea. Official dumpsites were located in the Bornholm Deep, Gotland Deep and Little Belt. These munitions contained chemical warfare such as blister agents (sulphur mustard), vomiting agents (Adamsite, Clark I, Clark II), tear gases (α-chloroacetophenone) and nerve gases (tabun).

The CHEMSEA project is an EU financed transnational collaboration including project partners and associated organizations, including governmental agencies and international organizations (www.chemsea.eu). The main focus of the project was to investigate the status of the dumping site at the Gotland Deep. Additionally, samples were collected at the known dumpsite at Bornholm Deep, a suspected dumpsite at Gdańsk Deep as well as at Gulf of Gdańsk and Słupsk Furrow. Figure 1 below shows the various official and suspected dumpsites of chemical warfare agents (CWA) in Baltic.

Figure 1. Known and suspected chemical munitions dumpsites in Baltic Sea.

1.2 Goal of the study

The objective of this project was to perform chemical analysis of sediment samples in connection with search and assessment of CWAs within the CHEMSEA Project. The analysis stage was preceded by literature review,[2] selection of target chemicals, planning of samples and analysis methods as well as an interlaboratory comparison test to verify the performance of the laboratories' analytical performance.[3]

The aim of the project is to obtain reliable analysis data, which can be compared between laboratories and to search for substances that will testify the presence of chemical weapons in the Baltic Sea. Using sophisticated methods such as optimised sample preparation, selected ion monitoring (SIM) and/or selected reaction monitoring (SRM) mass spectrometric techniques, quantification of most target chemicals at concentration level of 1–10 µg/kg dw (ppb) in sediment was deemed possible. Most of the methods used have been validated during earlier analysis by VERIFIN.

The data produced by the laboratories were compared with each other. The results has been collected by Polish Naval Academy (PNA) into a database. This database will be used in the characterization and mapping of suspected dumping areas and in an environmental risk assessment of the dumping areas.

1.3 Selection of target chemicals

Based on the information on dumping operations in Baltic,[4] several agents and related chemicals were selected as target chemicals. Some chemicals, e.g. hydrogen cyanide, were left out as they would not survive in analysable form in contact with water. The following agents were selected as the parent target chemicals:

- Sulphur mustard (**1**),
- Adamsite (**2**),
- Clark I (**3**),
- Triphenylarsine (**4**),
- Phenyldichloroarsine (**5**),
- α-Chloroacetophenone (**6**),
- Lewisite I (**7**) and
- Lewisite II (**8**).

Degradation products for these agents were as target chemicals based on the following factors:

- detected in previous studies or mentioned in literature
- can be expected to be found in aqueous conditions
- detectable with the available methods
- minimisation of number of analytes using derivatives common to many degradation products

The actual target chemicals including degradation products are summarised in Table 1. It should be noted that some chemicals were analysed only as degradation products at the parent chemicals are expected to degrade rapidly in contact with water (**2**, **3**, **5**, **7** and **8**). Some chemicals considered to be very stable were only analysed as the parent chemical (**4** and **6**).

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[2] Anders Östin, Review of Analytical Methods for the Analysis of Agents Related to Dumped Chemical Weapons for the CHEMSEA project, 2012. CHEMSEA report.

[3] Martin Söderström, Vesa Häkkinen and Olli Kostiainen, Inter-Calibration Study Results, 2014, CHEMSEA Deliverable D3.2.

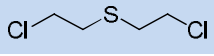
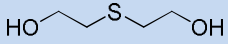
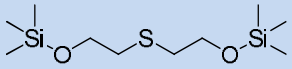
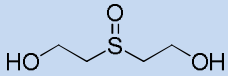
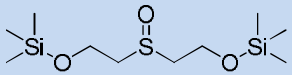
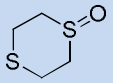
[4] "Report on Chemical Munitions Dumped in the Baltic Sea", Report of the Ad Hoc Working Group on Dumped Chemical Munition (HELCOM CHEMU), Danish Environmental Protection Agency, January 1994.

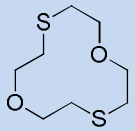
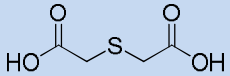
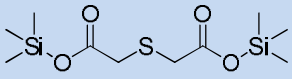
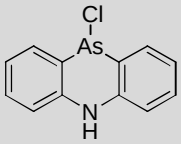
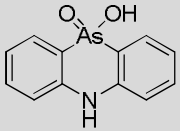
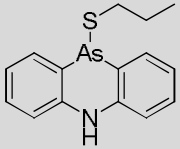
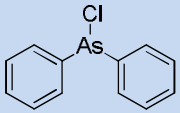
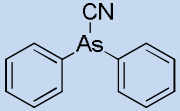
Many of the chemicals were analysed after derivatisation or oxidation, which make them easier to analyse with the selected analytical techniques. The following letters were used after the number of the chemical to identify the type of derivatisation/oxidation:

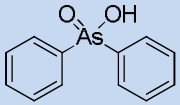
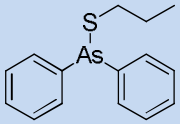
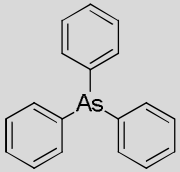
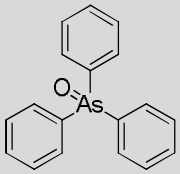
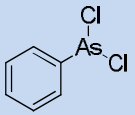
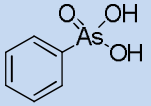
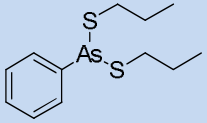
- **T** Derivatisation using propane-1-thiol (thiol derivative),
- **S** Derivatisation using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (silylation) or
- **O** Oxidation using hydrogen peroxide (H_2O_2).

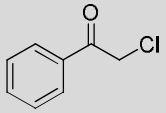
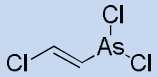
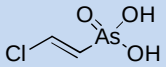
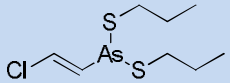
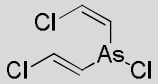
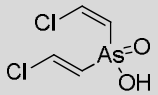
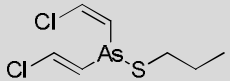
In total FOI had 23 target chemicals – 15 for gas chromatography-mass spectrometry (GC–MS) and eight for liquid chromatography-tandem mass spectrometry (LC–MS/MS). MUT analysed 16 target chemicals using gas chromatography-mass spectrometry (GC–MS/MS) only. VERIFIN analysed 21 target chemicals – 12 for GC–MS/MS and nine for LC–MS/MS. All but four chemicals screened by VERIFIN using LC–MS/MS were quantified.

Table 1. Summary of chemicals discussed in this report. Institutes analysing the chemicals are identified by codes: F = FOI, M = MUT and V = VERIFIN.

#	Chemical name (acronym) CAS	Structure	Description	GC-based			LC-based	
				intact	PrSH	BSTFA	intact	H_2O_2
1	Sulphur mustard (H) 505-60-2		Dumped CW agent	**FMV**				
1.1	Thiodiglycol (TDG) 111-48-8		Hydrolysis product of **1**				**V***	
1.1S	Bis(2-siloxyethyl)sulfide 20486-03-7		BSTFA derivative of **1.1**			**FM**		
1.1O	Thiodiglycol sulfoxide 3085-45-8		Oxidation product of **1** (either natural or with H_2O_2)					**FV**
1.1OS	Bis(2-siloxyethyl)sulfoxide 97916-03-5		BSTFA derivative of **1.1O**			**M**		
1.2	1,4-Dithiane 505-29-3		Degradation product of **1**	**FMV**				
1.2O	1,4-Dithiane oxide 19087-70-8		Oxidition product of **1.2**				**V***	
1.3	1,4-Oxathiane 15980-15-1		Degradation product of **1**	**FMV**				
1.4	1,4,5-Oxadithiepane 3886-40-6		Degradation product or by-product of **1**	**FMV**				
1.5	1,2,5-Trithiepane 6576-93-8		Degradation product or by-product of **1**	**FMV**				

#	Chemical name (acronym) CAS	Structure	Description	GC-based			LC-based	
				intact	PrSH	BSTFA	intact	H_2O_2
1.6	1,7-Dioxa-4,10-dithiacyclododecane 294-95-1		Degradation product or by-product of **1**	**FMV**				
1.7	Thiodiglycolic acid (TDGA) 123-93-3		Bacterial metabolite of **1.1**				**FV***	
1.7S	Bis(trimethylsilyl) 2,2'-thiodiacetate 20486-03-7		BSTFA derivative of **1.7**			**FM**		
2	*Adamsite (DM)* *578-94-9*		*Dumped CW agent*	***Not analysed as such***				
2O	5,10-Dihydrophenoarsazin-10-ol 10-oxide 4733-19-1		Oxidation product of **2** and all of its degradation products (either natural or with H_2O_2)					**FV**
2T	10-(propylthio)-5,10-dihydro-phenarsazinine 7269-24-1		Derivative of **2** and all of its degradation products		**F**			
3a	*Clark I (DA)* *712-48-1*		*Dumped CW agent. Also component in dumped arsine oil.*	***Not analysed as such***				
3b	*Clark II (DC)* *23525-22-6*		*(Possibly) dumped CW agent*	***Not analysed as such***				

#	Chemical name (acronym) CAS	Structure	Description	GC-based			LC-based	
				intact	PrSH	BSTFA	intact	H_2O_2
3O	Diphenylarsinic acid 4656-80-8		Oxidation product of **3a** and **3b** and all of their degradation products (either natural or with H_2O_2)					**FV**
3T	Diphenylpropylthioarsine 17544-92-2		Derivative of **3a** and **3b** and all of their degradation products		**FMV**			
4	Triphenylarsine (TPA) 603-32-7		Component in dumped arsine oil	**FMV**				
4O	Triphenylarsine oxide 1153-05-5		Oxidation product of **4** and all of its degradation products (either natural or with H_2O_2)	**M**				**FV**
5	*Phenyldichloroarsine (PDCA)* *696-28-6*		*Dumped CW agent. Also component in dumped arsine oil.*	***Not analysed as such***				
5O	Phenylarsonic acid 98-05-5		Oxidation product of **5** and all of its degradation products (either natural or with H_2O_2)					**FV****
5T	Dipropyl phenylarsonodithioite 1776-69-8		Derivative of **5** and all of its degradation products		**FMV**			

#	Chemical name (acronym) CAS	Structure	Description	GC-based			LC-based	
				intact	PrSH	BSTFA	intact	H_2O_2
6	α-Chloroacetophenone (CN) 532-27-4		*Dumped CW agent*	**FMV**				
7	*Lewisite I (L1)* *541-25-3*		*Dumped CW agent*	***Not analysed as such***				
7O	2-Chlorovinylarsonic acid 64038-44-4		Oxidation product of **7** and all of its degradation products (either natural or with H_2O_2)					**F**
7T	Dipropyl (2-chlorovinyl)arsonodithioite 677354-97-1		Derivative of 7 and all of its degradation products		**FMV**			
8	*Lewisite II (L2)* *40334-69-8*		*Dumped CW agent*	***Not analysed as such***				
8O	Bis(2-chlorovinyl)arsinic acid 157184-21-9		Oxidation product of **8** and all of its degradation products (either natural or with H_2O_2)					**FV**
8T	Bis(2-chlorovinyl) propylthioarsine 677355-04-3		Derivative of **8** and all of its degradation products		**FMV**			

* Target chemicals were screened instead of quantification (VERIFIN)
** Target chemicals were screened in pore water samples instead of quantification (VERIFIN)

1.4 Inter-calibration study

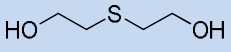
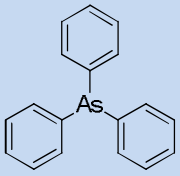
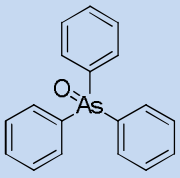
In order to test the performance of both the laboratories and the selected methods, an inter-calibration study was organised. Samples were sent to all five laboratories analysing sediment samples.[3]

Samples for CWA analysis was spiked with sulphur mustard related chemicals **1.1** and **1.2** as well as with triphenyl arsine (**4**) and its oxidarion product **4O**. A blank sample and spiked samples at two concentration levels were prepared (see Table 2). The stability of the chemicals in the samples was tested before sending the samples.

Based on the results of the inter-calibration study for the CWA analysis, it was seen that the laboratories could produce quite similar results. As the expected variation resulting from inhomogeneity is large in sediment samples, the differences between the laboratories' results were considered insignificant.

The results of the analysis of the low concentration sample are shown in Figure 2. There was a considerable difference in findings by laboratory C for 1,4-dithiane (1.2) – 30 µg/kg – compared to findings by laboratories A and B – both 100 µg/kg. The delivery of the sample package for laboratory C took 16 days due to the courier company. When the samples were finally received, they had been thawed for a long time. After further testing, it was noticed that 1,4-dithiane did, indeed, disappear from the sample. The probable cause for this was its oxidation into 1,4-dithiane oxide (**1.2O**). Other chemicals seemed to be stable in the sample.

Table 2. Chemicals selected for the inter-calibration study.

#	Chemical name	CAS Number	Structure	Spiking levels	
				Low	High
1.1	Thiodiglycol	111-48-8		270 µg/kg	2700 µg/kg
1.2	1,4-Dithiane	505-29-3		670 µg/kg	6700 µg/kg
4	Triphenylarsine	603-32-7		170 µg/kg	1700 µg/kg
4O	Triphenylarsine oxide	1153-05-5		67 µg/kg	670 µg/kg
–	Arsine in nitric acid (2–3%)	7440-38-2	AsH_3	75 mg/kg	200 mg/kg

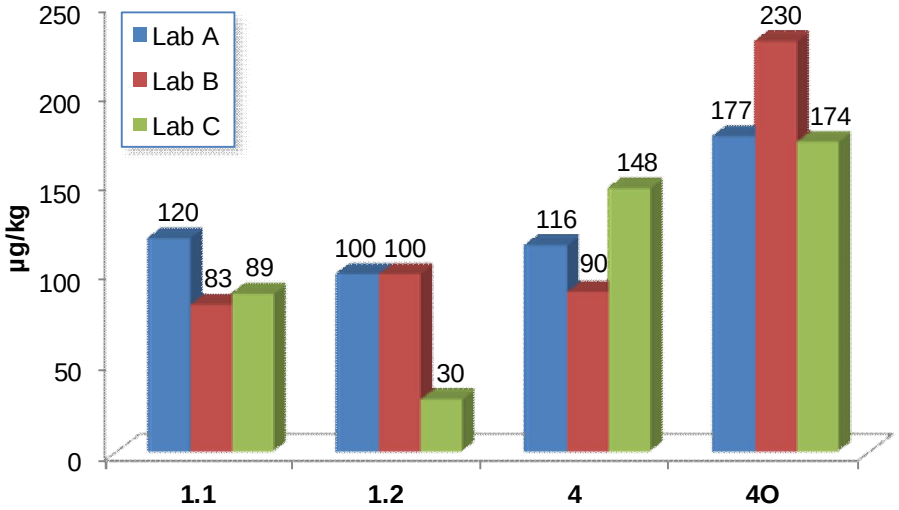


Figure 2. Comparison of CWA analysis results for low concentration CWA samples by different laboratories. The delivery of samples for laboratory C took 16 days to deliver. In tests, it was noticed that 1,4-dithiane (**1.2**) did, indeed, disappear from the sample. The probable cause for this was its oxidation to 1,4-dithiane oxide (**1.2O**).

The laboratories performing the arsenic analysis received samples containing both the organic CWA chemicals as well as inorganic arsenic as arsine (AsH_3) at much higher concentration. The concentrations of inorganic arsenic were 75 and 200 mg/kg in the low and high concentration samples, while the organic arsenic was 1.2 and 12 mg/kg.

The total arsenic results from laboratories C and D were matching well with the spiking levels. The value of the inorganic arsenic, which is used to calculate the concentration of organic arsenic was more variable. This meant that the values for organic arsenic are quite meaningless as they can easily be more than ten times the real value.

Although laboratory E got too low total arsenic values, it was decided that samples would be sent to all laboratories. The actual results then compared between laboratories to verify the validity of the analysis (see chapter 3.4.2).

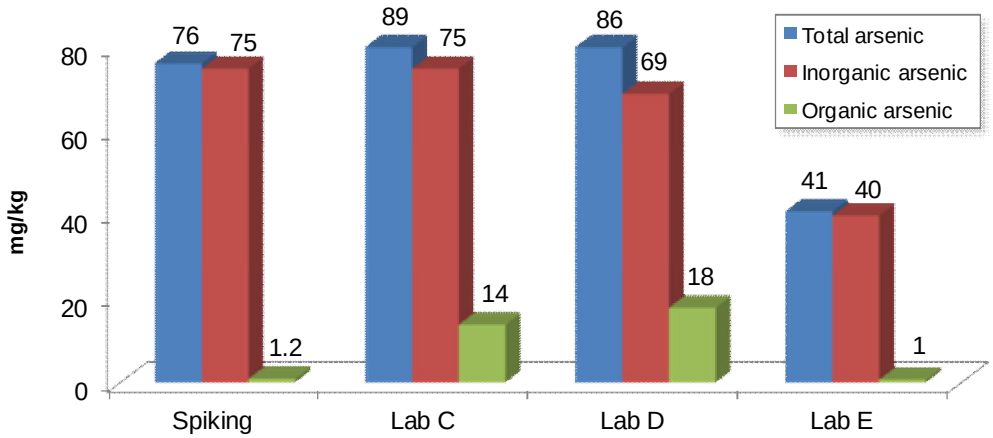


Figure 3. Comparison of arsenic analysis results for low concentration arsenic samples by different laboratories.

2. Experimental

2.1 Reference materials

One of the basic principles behind the design of the analysis methods was to use targeted analysis in order to achieve the best sensitivity. As mentioned above, the goal was to reach concentration level of 1–10 µg/kg dw (ppb) in sediment.

To build a targeted analysis method, reference materials are required for development optimisation of the method as well as for the testing of the performance of the analysis method.

All three laboratories had reference chemicals available for all target chemicals they analysed. The reference chemicals available in each laboratory are summarized in Table 3.

Some reference chemicals were synthesised in the laboratories. VERIFIN had received some of the chemicals from its cooperation laboratories (Finish Defence Forces Technical Research Centre and Spiez Laboratory, Switzerland). Part of the reference chemicals were available commercially.

Some of the chemicals were not available as pure standards. These were created in the sample preparation from other reference chemicals. These chemicals belong into the following categories:

- trimethylsilyl derivatives
- propan-1-thiol derivatives
- oxidized products

One advantage of using the reaction to produce the reference material is that the correct performance of the sample preparation procedures is constantly tested by analysis of calibration and control samples in which reference chemicals are spiked.

Table 3. Summary of used reference chemicals during the sample analysis. Used symbols: in-house synthesis (■), commercial chemical (●), received from another laboratory (▲), produced in a reaction during sample preparation (R), not analysed (–).

Chemical		FOI	MUT	VERIFIN
1	Sulfur mustard	■	■	▲
1.1	Thiodiglycol	●	●	●
1.1S	Bis(2-siloxyethyl)sulfide	R	R	–
1.1O	Thiodiglycol sulfoxide	■	■	R
1.1OS	Bis(2-siloxyethyl)sulfoxide	–	R	–
1.2	1,4-Dithiane	■	●	●
1.2O	1,4-Dithiane 1-oxide	–	■	●
1.3	1,4-Oxathiane	●	●	▲
1.4	1,4,5-Oxadithiepane	■	■	▲
1.5	1,2,5-Trithiepane	■	■	▲
1.6	1,7-Dioxa -4,10-dithiacyclododecane	■	■	▲
1.7	Thiodiglycolic acid	●	●	●
1.7S	Bis(trimethylsilyl) 2,2'-thiodiacetate	R	R	–
2	Adamsite	■	■	■
2O	5,10-Dihydrophenoarsazin-10-ol 10-oxide	●	■	R
2T	10-(propylthio)-5,10-dihydrophenarsazinine	R	R	R
3a	Clark I	■	■	■
3O	Diphenylarsinic acid	R	■	R
3T	Diphenylpropylthioarsine	R	R	R
4	Triphenylarsine	●	●	●
4O	Triphenylarsine oxide	●	●	R
5	Phenyldichloroarsine	■	■	■
5O	Phenylarsonic acid	●	■	R
5T	Dipropyl phenylarsonodithioite	R	R	R
6	α-Chloroacetophenone	■	■	●
7	Lewisite I	■	■	▲
7O	2-Chlorovinylarsonic acid	R	■	R
7T	Dipropyl (2-chlorovinyl)arsonodithioite	R	R	R
8	Lewisite II	■	■	▲
8O	Bis(2-chlorovinyl)arsinic acid	R	■	R
8T	Bis(2-chlorovinyl) propylthioarsine	R	R	R

2.2 Division of samples between laboratories

As there were three laboratories – FOI, MUT and VERIFIN – performing the analysis of agents and their degradation products, the workload of the analysis was divided between the laboratories.

However, in order to be able to compare the laboratories' results it was decided to send portion of each sample to two laboratories if possible. Some of the samples were too small to be split between laboratories. Additionally, sample portions were send to three laboratories for analysis of arsenic: IOPAN, MUT and LEPA.

In total 178 samples were sent to organic analysis. Of these, 78 samples were sent to only one laboratory, 84 to two laboratories and one sample to all three laboratories. The plan was to distribute the samples evenly between the three laboratories, but, due to practical reasons, there were differences in the number of received samples.

FOI received 89 sample (31 of these were one-portion samples), MUT received 73 (20) and VERIFIN received 87 (27). From these samples they selected some for pore water sample analysis (FOI 11 samples, MUT 10 and VERIFIN 10).

Three laboratories performed analysis of the arsenic concentration in sediment samples: IOPAN, MUT and LEPA. The analyses for LEPA were done in a subcontracting laboratory (Nature Research Center of the Institute of Geology and Geography). In total, arsenic measurement was performed on 180 sediment samples. Some sediment samples were analysed only for arsenic i.e. no analysis of CWA. IOPAN analysed all but two of the samples. MUT analysed 44 samples and LEPA 59 samples. In total, 88 samples were analysed by two laboratories and six samples by all three laboratories.

2.3 Sample Preparation for CWA analysis

All sediment samples were received frozen by the analysis laboratories. The laboratories kept the samples in freezer until the start of the analysis.

After thawing the samples, a subsample (ca. 10 g) was taken into analysis and the rest of the sample was refrozen. The subsample was centrifuged to separate the sediment and pore water from each other. The pore water samples were stored for possible reanalysis.

2.3.1 Sediment samples

Many of the target chemicals, including the intact agents, are non-polar and require a non-polar extraction solvent. On the other hand many of the target chemicals are very polar as they are both hydrolysis and oxidation products of the agents. Figure 4 presents the behaviour of the target chemicals after degradation and sample preparation.

All polar chemical chemicals require derivatisation before GC–MS analysis. Arsenic-containing chemicals can be derivatised using propane-1-thiol. Hydrolysis products, on the other hand, can be derivatised using BSTFA.

Some of the polar degradation products can be analysed directly by LC–MS. Some do not ionise well as such but do so after oxidation with hydrogen peroxide.

Based on the above principles, one can summarise the behaviour of the target chemicals during the sample preparation and the proper analysis techniques for each analyte. See Figure 5for the summary of sample preparation of sediment samples.

The combined sample preparation scheme is shown in Figure 5. The scheme has been simplified to show only the major steps.

Full details of the sample preparation methods can be found in the individual laboratory reports of FOI, MUT and VERIFIN in Attachments 3–5.

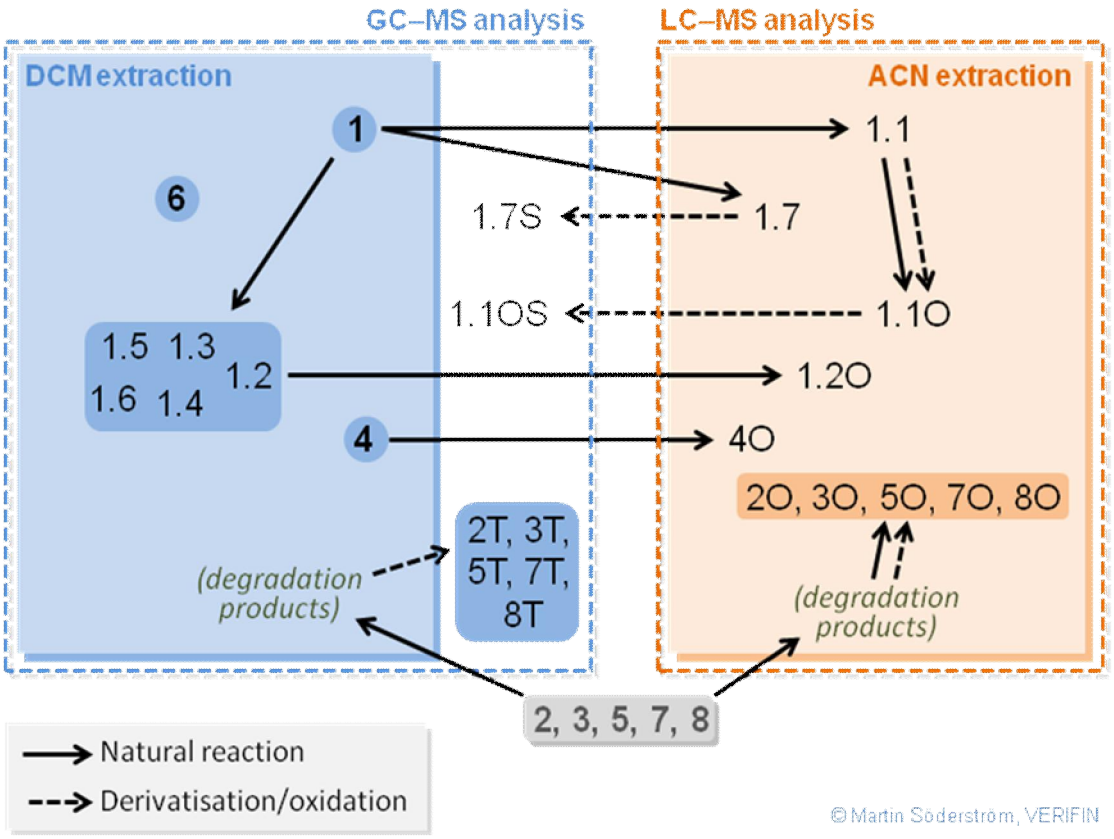


Figure 4. Behaviour of the target chemicals in sample preparation and the applied analysis methods.

2.3.2 Pore water samples

Only part of the pore water portions were selected for analysis. The selection of the possibly interesting samples was made after the sediment results (shown in Table 3) were available.

All three laboratories approached the pore water samples differently. The reason for this is partly that the methods for pore water analysis are harnonized like the methods for sediment analysis.

FOI derivatised part of the pore water directly using propan-1-thiol for GC–MS analysis. The other part they cation exchanged and oxidised for LC–MS/MS analysis.

VERIFIN performed the cation exchange, but analysed one part of the eluate intact and other part after oxidation using LC–MS/MS. They did not perform the GC–MS/MS analysis for pore water sample.

MUT extracted part of the pore water with dichloromethane. This sample they analysed both intact and after derivatisation using propan-1-thiol using GC–MS/MS. The other part of the pore water was then evaporated to dryness and analysed it after derivatisation with either propan-1-thiol or BSTFA using GC–MS/MS.

The sample preparation scheme is summarised in Figure 5.

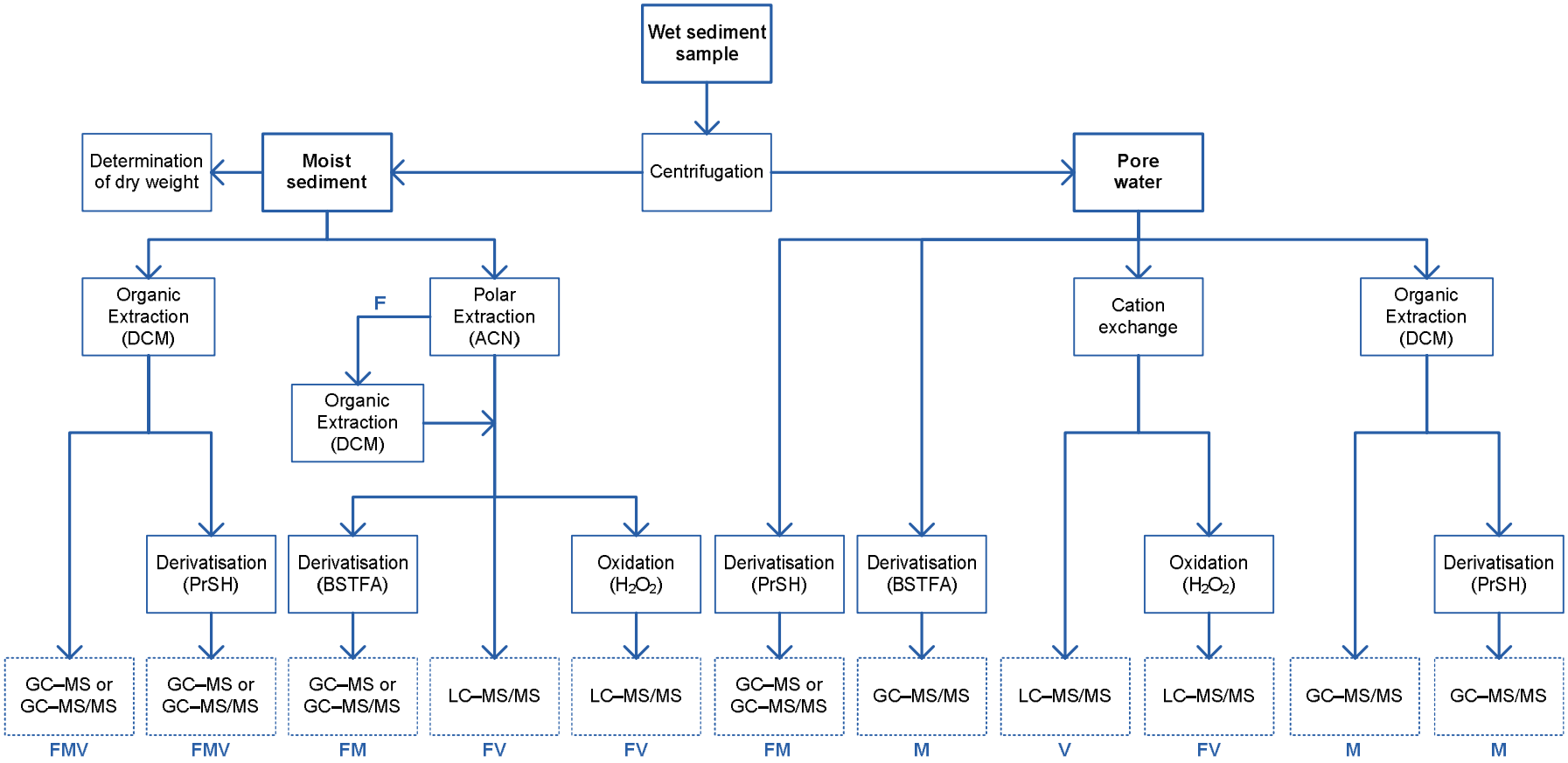


Figure 5. Summary of the sample preparation schemes by the different laboratories: F = FOI, M = MUT and V = VERIFIN.

2.4 Sample preparation for arsenic analysis

Two different sample preparation procedures were carried out for the arsenic analysis: total arsenic and inorganic arsenic. The idea behind using two methods was that by subtracting inorganic arsenic from total arsenic, the organic arsenic present in the samples could be calculated. Hypothesis was that this could possibly be used to estimate CWA levels in the sediment. However, as seen from the results of the inter-calibration study (chapter 1.4), the organic arsenic values obtained do not represent the real situation and cannot be for estimation of CWA concentrations.

Before the sample analysis, IOPAN let the samples to thaw for 24 h. The samples were then homogenized, freeze-dried and homogenized again. Homogenization was not necessary for arsenic measurements, but IOPAN was also performing other heavy metal analysis in the same samples.

2.4.1 Total arsenic measurement

IOPAN performed dry digestion in a muffle oven (at 550 °C for 2h) using $Mg(NO_3)_2$. The digested sample was diluted with MilliQ water and reduced with hydrochloric acid (HCl) and potassium iodide (KI). This converted all arsenic into As(III). The recovery of the procedure was 99.6 %.

LEPA's subcontractor performed the total arsenic measurement according to standard LST EN 16206, which is for arsenic determination in animal feed stuffs. The digestion of the samples was done by dry digestion at 575 °C overnight in presence of magnesiums oxide and magnesium nitrate. The digested sample was dissolved in 6 M HCl. The solution was heated, filtered diluted with 3 M HCl. The solution was reduced using KI and ascorbic acid.

2.4.2 Inorganic arsenic measurement

The idea behind the measurement of inorganic arsenic was to extract the inorganic arsenic from the sediment and get rid of organic arsenic in the process. This way the amount of removed organic arsenic could be calculated by subtracting the amount inorganic arsenic from the total arsenic. As already discussed in chapter 1.4 (Inter-calibration study), the total arsenic measurement was considered acceptable, but the there were doubts on performance of the extraction in removal of organic arsenic chemicals from the samples.

At IOPAN, the sediment was first extracted four times using 30 % HCl and twice with MilliQ water. The combined extract was reduced using KI at 60 °C for 30 min and adjusted to HCl concentration of more than 9M. This solution was extracted four times with chloroform. The organic extract was then extracted twice with milliQ water. This water phase was reduced using HCl and KI and analysed for inorganic arsenic. The recovery of this method was 94.2 %.

LEPA's subcontractor performed the inorganic arsenic measurement according to standard EN 16278:2012, which is for inorganic arsenic determination in animal feed stuffs. The samples were extracted using diluted HCl and hydrogen peroxide with microwave assisted heating at 90 °C. In the process, As(III) was oxidised to As(V). The extract was centrifuged, diluted ammonium carbonate and adjusted to pH 6.5. The Solution was loaded into strong anion exchange SPE cartridges, washed with 0.5 M acetic acid and eluted with 0.4 M HCl. The eluate was reduced [As(V) reduced back to As(III)] using KI and ascorbic acid.

2.5 Mass spectrometric methods

Each of the three laboratories have slightly different instrumention in their use. Still, the basic principles and the analysis methods are very similar. Depending on the instrumentation the sample preparation was modified to get the maximal performance from each laboratory's capacity.

2.5.1 GC–MS analysis

All laboratories performed gas chromatography-electron ionisation mass spectrometric (GC–EI/MS) analysis. FOI used a single quadrupole GC–EI/MS instrument in selected ion monitoring (SIM) mode, while both MUT and VERIFIN used triple quadrupole GC–EI/MS/MS instruments in selected reaction monitoring (SRM) mode.

All laboratories used three GC–EI/MS ions or three GC–EI/MS/MS transitions per chemical for the quantitation and identification of the chemicals.

For some transitions, MUT used so-called pseudo-SRM at low collision energies instead of pure SRM. In this method, the same ion is selected as both precursor and product ion. The method does not have the selectivity of pure SRM as it is more like SIM done on single quadrupole instruments.

Typically, GC–MS analysis suffered more from interferences in the sample background. Based on the limit of quantitation (LOQ) data in Table 4, the sensitivity difference between GC–MS and GC–MS/MS method was typically 1–10 fold, but could in some extreme cases be even 30–100 fold. The difference between GC–MS/MS in MUT and VERIFIN was typically from 0.7 to 6 fold.

2.5.2 LC–MS analysis

FOI and VERIFIN also performed LC–MS/MS analysis using a triple quadrupole instrument. FOI used electrospray ionisation (ESI; mostly in positive ion mode) and VERIFIN used atmospheric pressure chemical ionisation (APCI; positive ion mode). Both laboratories used UHPLC-type liquid chromatography. The differences in LOQ values were 0.2–4 fold.

Both laboratories used two transitions per chemical for the quantitation and identification of the chemicals.

2.5.3 Validation of the applied methods

Each of the laboratories measured both calibration samples and control samples during the analyses. The LOQ values were determined based on these runs.

If both relative error and relative standard deviation of the lowest calibration standard was below 35 %, the calibration standard was accepted as the LOQ. If there were problems with the lowest standard, the next lowest one fulfilling the criteria was accepted.

The used method does not give the lowest possible LOQ, but it gives a guarantee that the data obtained is valid. As sediment samples can be very different depending on the sampling area, the used approached also compensates for the possible differences in the behaviour of the target chemicals in different sediment matrices.

The validation data can be found in the individual laboratory report in Attachments 3–5, but the LOQ values are summarized in Table 4 below.

The lowest acceptable standard gives the practical LOQ during the analysis, but by using the standard deviation of the lowest acceptable standard multiplied by ten, a more realistic value for LOQ can be obtained. Also these values are given in Table 4.

Table 4. Limits of quantification (LOQ) of the target analytes in sediment samples. Value in Std column is the lowest accepted standard. Value in the *10σ* column is the LOQ calculated from the standard deviation of the lowest accepted standard. All values presented in µg/kg dw.

Chemical	GC–MS		GC–MS/MS				LC–MS/MS			
	FOI		MUT		VERIFIN		FOI		VERIFIN	
	Std	10σ	Std	10σ	Std	10σ	Std	10σ	Std	10σ
1	1.1	3	0.31	0.3	0.45	0.3				
1.1									screening	
1.1S	2.2	3	0.31	1.3						
1.1O							11	38	8.8	18
1.1OS			6.3	1.9						
1.2	11	14	0.31	1.0	0.45	0.15				
1.2O									screening	
1.3	1.1	3	3.1	1.1	0.45	0.15				
1.4	2.2	3	1.6	0.9	2.3	5.1				
1.5	2.2	6	1.6	1.0	2.3	6.7				
1.6	nd (sed.)		6.3	21	0.45	0.6				
1.7							nd (sed.)		nd (sed.)	
							1.4*	2*		
1.7S	11	3	6.3	6.3						
2O							1.4	1	4.4	4.3
2T	nd (sed.)									
3O							1.4	2	8.7	4.3
3T	11	15	13	6.5	2.1	3.2				
4	2.2	6	1.6	0.5	2.3	3.6				
4O			3.1	1.8			nd (sed.)		4.4	18
							1.4*	3*		
5O							1.4	1	screening	
5T	11	18	3.1	1.4	2.1	2.1				
6	22	49	3.1	1.4	0.45	1.5				
7O							nd (sed.)			
							1.4 (s)	2 (s)		
7T	11	32	3.1	5.8	2.1	2.0				
8O							5.4	8	2.2	4.3
8T	2.2	7	1.6	2.6	2.1	6.7				

screening = screening only; LOQ not determined
nd (sed.) = not detected in after spiking into sediment extracts
* = listed LOQ has been measured after spiking into solvent

2.5.4 Criteria for identification and quantitation

The analysis results consist of two parts: identification of the chemical and the determination of its concentration in the samples. Criteria were defined for both tasks in order to make the results reliable and comparable between laboratories.

The identification criteria are based on those defined by the European Commission:[5]

- The retention time of the analyte should be within acceptable tolerance compared to the reference standard. Tolerance for GC-based methods is ±0.5 % and for LC-based methods is ±2.5 %.
- Always at least two ions/transitions must be measured for each analyte and the ratio(s) of the ions/transitions should with acceptable tolerances compared to the reference standard. Tolerances depend on both technique and the reference standard ion/transition ratio:
  - GC–EI/MS: for ratios >50 % tolerance is ±10 %, for >20–50 % ±15 %, for >10–20 % ±20 % and for ≤10 % ±50 %
  - GC–EI/MS/MS and LC–MS/MS: for ratios >50 % tolerance is ±20 %, for >20–50 % ±25 %, for >10–20 % ±30 % and for ≤10 % ±50 %

Examples of the use of the identification criteria can be e.g. in Figure 6 on page 32.

For quantitation, common criteria for acceptance of the calibration standards were agreed before the start of the actual analyses. Each laboratory used series of calibration standards to create a calibration curve. In order to accept a calibration point, the error and relative standard deviation of the measurement values must remain below 35 %. This data is presented in analysis reports of each laboratory in Attachments 3 to 5. The summary of the lowest acceptable calibration standards is shown in Table 4 on page 21.

2.6 Arsenic analyses

IOPAN performed the arsenic analyses using hydride vapour generation atomic absorption spectroscopy (HVG-AAS). All samples were analysed in triplicate.

The limit for the detection was 0.03 mg/kg. The mean standard error was 0.31 mg/kg for the total arsenic measurements and 0.44 mg/kg for the inorganic arsenic measurements.

In addition to arsenic, IOPAN measured concentrations of heavy metals (Cr, Mn, Fe, Cu, Pb, Cd, Zn, Co and Ni) as well as estimated the contents of the organic matter and analyzed the sediment grain size.

MUT used graphite furnace atomic absorption spectroscopy (GF-AAS) for their analysis.

Analysis for LEPA were performed by Nature Research Center of the Institute of Geology and Geography. They used similar HVG-AAS instrument as IOPAS. The method limit of detection was 0.10 mg/kg.

IOPAN and MUT provided values for total arsenic, inorganic arsenic and calculated organic arsenic for all samples they analysed. LEPA provided this data for eight samples and only total arsenic values for the rest of the samples.

[5] Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC), *Official Journal of the European Communities*, 2002, 45 (L221) 8–36.

3. Results and discussion

3.1 Results of the CWA analysis

3.1.1 Sediment samples

In total, the three laboratories performing the analysis analysed aliquots from 175 sediment samples. Of these, 55 samples (31 %) were positive i.e. contained at least one target chemical. The highest found concentration was 1700 µg/kg. The summary of positive samples is presented in Table 5. The full results of the sediment analysis are shown on a map in Attachment 1 and summarised in tabular form in Attachment 2.

Two intact CWA chemicals were found: sulphur mustard (**1**) and α-chloroacetophenone (**6**). Additionally, triphenylarsine (**4**) was found both as such and after oxidation (as **4O**). Degradation products were found for sulphur mustard (**1**), Adamsite (**2**), Clark I (**3**) and phenyldichloroarsine (**5**). The only target chemicals, which were not detected were Lewisite I (**7**) and Lewisite II (**8**).

The results are further discussed below by chemical (chapter 3.2) and by area (chapter 3.3).

3.1.2 Pore water samples

Based on the results of the sediment samples, 23 pore water portions of sediment samples were selected for pore water analysis. Out of these samples, 18 were positive. The results of the pore water analysis are presented in Table 6.

The assumption for the selection of the samples for pore water analysis was that a high concentration in sediment would possibly result in transfer of the chemical into the pore water. Table 7 presents the transfer of the chemicals from sediment to pore water.

The most remarkable finding in the pore water analysis is that degradation products of Clark (analysed as **3O** and **3T**) seem to be transferring to pore water in relatively high amounts. The highest detected concentration of **3O** in pore water was 940 µg/l. This could mean that these chemicals could be more readily available for transfer into surrounding sea water and therefore likely to spread to the neighbouring areas and/or to transfer to organisms close to the contaminated area.

The transfer of 1,4,5-oxadithiepane (**1.4**) from sediment to pore water seems to be also quite high, but there is little data for this. Also, this chemical is likely to cause less problems in environment that the arsenic-containg degradation products of Clark.

3.1.3 Sediment core samples

In order to evaluate the distribution of the CWA below surface, Finnish Environment Institute (SYKE) took nine sediment core samples. These samples were cut in either 2.5 or 5 cm slices. Three core samples were sent to each of the laboratories performing CWA analysis.

The results of these analyses were quite disappointing: only two cores analysed by MUT were found to contain target chemicals. Two chemicals – both degradation products of sulphur mustard – were detected in these samples: thiodiglycol (**1.1**, measured as **1.1S**) in one slice of core sample *Q_7466* and five slices of sample K_1857 in concentrations from 18 to 53 µg/kg dw as well as 1,4-dithiane (**1.2**) in six slices of core sample *Q_7466* in low concentrations (0.3–1.7 µg/kg dw) .

As these core samples were the only samples were thiodiglycol was detected, MUT verified the correctness of the findings by repeating some of the analysis. The results are discussed under each chemical in chapter 3.2 (Findings by chemical warfare agent).The reanalysis results of these samples are presented in chapter 3.4.2 (Sediment core samples by MUT).

3.1.4 Toxicity of degradation products

The target chemicals found in both sediment and pore water portions of the sediment samples were mainly degradation products. Their concentrations vary from low ppb-levels (µg/kg dw or µg/l) to low-ppm levels (mg/kg or mg/l). Typically, the degradation products are less toxic than the respective agents, but their toxic effects have not been widely studied.

Degradation products of sulphur mustard (**1**) seem relatively non-toxic 1,4-dithiane (**1.2**) has LD_{50} value of ca. 3.5 g/kg (in rats)[6]

However, arsenic-containing degradation products can be much more toxic than those of sulphur mustard. Diphenylarsinic acid (**3O**) has been found to cause both developmental and acute problems in rats at drinking water levels of 20 mg/l.[7] In Japan, severe health problems – including cerebral symptoms – have been characterized due to well water contaminated by **3O**.[8] The concentration of **3O** in the contaminated well was found to be 4.5 mg/l. The LD_{50} value has been reported to be 17 mg/kg (in mice).[9]

Based on the above values, degradation products of sulphur mustard do not seem to pose any clear risk to marine organisms or humans as such. **However, the detected levels of arsenic-containing chemicals are quite close to those found to cause severe health effects.**

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6 G. Reddy and D.A. Mayhew, "Acute Oral Toxicity (LD50) Study in Rats with Dithiane", *Int. J. Toxicol.*, **11** (1992) 667.

7 T. Negishi, Y. Matsunaga, Y. Kobayashi, S. Hirano and T. Tashiro, "Developmental subchronic exposure to diphenylarsinic acid induced increased exploratory behavior, impaired learning behavior, and decreased cerebellar glutathione concentration in rats", *Toxicol. Sci.*, September 5, 2013, *doi: 10.1093/toxsci/kft200.*

8 K. Ishii, A. Tamaoka, F. Otsuka, N. Iwasaki, K. Shin, A. Matsui, G. Endo, Y. Kumagai, T. Ishii, S. Shoji, T. Ogata, M. Ishizaki, M. Doi, and N. Shimojo, "Diphenylarsinic Acid Poisoning from Chemical Weapons in Kamisu, Japan", *Ann. Neurol.*, **56** (2004) 741–745.

9 J. Marhold, Přehled průmyslové toxikologie: Organické látky, Avicenum, Prague 1986, 1700 pages, page 1276.

Table 5. Summary of the sediment findings by FOI, MUT and VERIFIN. Results presented in µg/kg dw.

Sample code	Lab	H							DM	DA		TPA		PDCA		CN	Sampling area	Comment by sampler
		1	1.1OS	1.2	1.3	1.4	1.5	1.7S	2O	3O	3T	4	4O	5O	5T	6		
WH349/B13/1	MUT										25						Bornholm Deep	van Veen sampler
	VERIFIN								17									
WH349/B13/2	FOI								4									van Veen sampler
	MUT					22	2.3								17			
WH349/B13/3	FOI					4.2	5		1400	10								van Veen sampler
	VERIFIN	<LOQ*		0.52		2.8	28		210		150	8.0			4.0			
WH349/B13/4	VERIFIN						0.82											van Veen sampler
WH349/B13/6	MUT														27			van Veen sampler
WH349/B13/7	VERIFIN						1.1			68	19	5.5			41			van Veen sampler
WH349/B13/8	FOI								2.5	2.6								van Veen sampler
WH349/B13/10	VERIFIN						0.65											van Veen sampler
1Apr13ROV	FOI					8.5	8.7			2.6								"wreck3, but no signs of wreck", ROV sample
2Apr13ROV	VERIFIN								22	1300			190					"Hot As", ROV sample
3Apr13BOX	FOI					14	35		312	1700	288	1200	200		480			"Hot As", Box corer
	VERIFIN								5.2	1300			180					
3Apr13ROV	MUT						9.2			900		350	590			7.5		"Hot As", ROV sample
4Apr13ROV	MUT					10	7.5				240		99					"HotYperite", ROV sample

Sample code	Lab	H							DM	DA		TPA		PDCA		CN	Sampling area	Comment by sampler
		1	1.1OS	1.2	1.3	1.4	1.5	1.7S	2O	3O	3T	4	4O	5O	5T	6		
5Apr13ROV	VERIFIN								30	210			67				Bornholm Deep	“Hot Yperite”, ROV sample
6Apr13ROV	FOI					6	18		51	450	74	30		1300	480			“Hot Yperite”, ROV sample
6Apr13BOX	MUT			2.1			9.0				490	33	35		99			“Hot Yperite”, Box corer
	VERIFIN								30	35								
7Apr13ROV	FOI								4.6	5.1	30							”supposed to be a wreck…no signs”, ROV sample
7Apr13BOX	MUT						0.8					20	62		45	7.0		”supposed to be a wreck…no signs”, Box corer
	VERIFIN								6.0									
ROV9	MUT				3.7						35						Gotland Deep	“suspicious”, ROV sample
ROV11	VERIFIN						0.60											“suspicious”, ROV sample
ROV15	VERIFIN														5.3			“suspicious”, ROV sample
11Mar12	VERIFIN			0.9														Box corer
13Mar12	VERIFIN														3.2			Box corer
6GTapr12	MUT										13							Box corer
11GTapr12	MUT					12												Box corer
13GTapr12v2	VERIFIN											1.2						Box corer
1ROVfeb13+30m	MUT					20												ROV sample

Sample code	Lab	H							DM	DA		TPA		PDCA		CN	Sampling area	Comment by sampler
		1	1.1OS	1.2	1.3	1.4	1.5	1.7S	2O	3O	3T	4	4O	5O	5T	6		
10ROVfeb13+20m	MUT			45		20											Gotland Deep	ROV sample
11ROVfeb13	MUT				0.3													ROV sample
15ROVfeb13	MUT					33												ROV sample
17feb13	FOI									21				19				Mines, shells in close vicinity, Box corer
21ROVfeb13	MUT										22							Mines, shells in close vicinity, ROV sample
24feb13	MUT						1.3											Box corer
23ROVfeb13+30m	FOI									0.8								ROV sample
27ROVfeb13	FOI								2.9	1.6								ROV sample
28ROVfeb13	MUT					21												ROV sample
10apr13ROV	FOI								11	200				19				ROV sample
	MUT										25							
13apr13ROV	FOI									2.1								Bomb, ROV sample
14apr13BOX	MUT										24	250						Box corer
18apr13BOX	MUT					12												Box corer
20apr13BOX	MUT						0.5											Box corer
CWA2	FOI										17	14			42			
WH349/B15/2	MUT							550			20	97					Gdansk Deep	van Veen sampler
ROV3	MUT							533			240	27						ROV sample
ROV4	MUT		200			40												“Wreck”, ROV sample

Sample code	Lab	H							DM	DA			TPA		PDCA		CN	Sampling area	Comment by sampler
		1	1.1OS	1.2	1.3	1.4	1.5	1.7S	2O	3O	3T	4	4O	5O	5T	6		
ROV7	MUT			7.1			2.3											Gdansk Deep	“suspicious”, ROV sample
7Mar12	VERIFIN											61							Box corer
4GDapr12	MUT														570				Box corer
7GDapr12	MUT										54	200	84						Box corer
11GDapr12	MUT		260												89				Box corer
1mar12	MUT			25														Gulf of Gdańsk	Box corer
3SEP12	MUT		260																van Veen sampler
5SEP12	VERIFIN											0.7							“suspicious”, van Veen sampler
4R3RS	MUT					9.8									150			Słupsk Furrow	Box corer
8R3RS	MUT					21									79				Box corer
WH349/B09/2	MUT		610		122	19						20			20			Reference Area	van Veen sampler

* The sulphur mustard finding in sample WH349/B13/3 was well below quantitation limit, but it was identified unambiguously *(estimated amount in the sample was 0.030 µg/kg dw).*

Table 6. Summary of samples for which the pore water fractions were analysed. The cells with results matching with pore water findings are highlighted.

Sample code	Lab	Pore water fraction (µg/l)									Sediment fraction (µg/kg dw)								
		H		DM	DA		TPA		PDCA		H		DM	DA		TPA		PDCA	
		1.4	1.5	2O	3O	3T	4	4O	5O	5T	1.4	1.5	2O	3O	3T	4	4O	5O	5T
WH349/B09/2	MUT	19									19		–	–		20		–	20
WH349/B13/3	VERIFIN	–	–	4.2		–	–			–			210		150	8.0			4.0
	FOI			17							4.2	5	1400	10					
WH349/B13/7	VERIFIN	–	–		12	–	–			–				68	19	5.5			41
WH349/B15/2	FOI																		
	MUT					5							–	–	20	97		–	
3SEP12	MUT					47	4						–	–				–	
4SEP12	VERIFIN	–	–			–	–			–									
7Mar12	VERIFIN	–	–			–	–			–									
4GDapr	FOI																		
7GDapr12	FOI																		
	MUT					6							–	–	54	200	84	–	
11GDapr12	VERIFIN	–	–			–	–			–									
10ROVfeb13+20m	FOI																		
17feb13	FOI													21				19	
2Apr13ROV	VERIFIN	–	–		159	–	–		+*	–			22	1300			190		

Sample code	Lab	Pore water fraction (µg/l)									Sediment fraction (µg/kg dw)								
		H		DM	DA		TPA		PDCA		H		DM	DA		TPA		PDCA	
		1.4	1.5	2O	3O	3T	4	4O	5O	5T	1.4	1.5	2O	3O	3T	4	4O	5O	5T
3Apr13BOX	VERIFIN	–	–		601	–	–	12	+*	–			5.2	1300			180		
	FOI	1.8	3.4		940	250	68	20	4		14	35	310	1700	290	1200		20	210
3apr13ROV	MUT					10	3					9.2	–	–	900	350	590	–	
5Apr13ROV	VERIFIN	–	–			–	–		+*	–			30	210			67		
6Apr13BOX	VERIFIN	–	–			–	–			–			30	35					
	MUT					6						9.0	–	–	490	33	34	–	45
6Apr13ROV	FOI				0.7			0.1			6	18	51	450	74	30		1300	480
10Apr13ROV	FOI												11	200				19	
	MUT					6	1						–	–	25	250		–	
14Apr13BOX	VERIFIN	–	–			–	–			–									
	MUT					6							–	–	24			–	
ROV3	FOI																		
	MUT						1	2					–	–	54	200	84	–	
ROV9	MUT					7							–	–	35			–	
CWA2	FOI														17	14			42

* The oxidation product for PDCA (**5O**) was screened only

Table 7. Ratio of pore water findings to sediment findings pore water. Values calculated by dividing pore water values (µg/l) by sediment values (µg/kg) shown in Table 6.

Sample code	Lab	H		DM	DA		TPA		PDCA
		1.4	1.5	2O	3O	3T	4	4O	5O
WH349/B09/2	MUT	100 %							
WH349/B13/3	VERIFIN			2.0 %					
	FOI			1.2 %					
WH349/B13/7	VERIFIN				17 %				
WH349/B15/2	MUT					25 %			
3SEP12	MUT					*	*		
7GDapr12	MUT					11 %			
2Apr13ROV	VERIFIN				12 %				*
3Apr13BOX	VERIFIN				46 %			6.7 %	*
	FOI	13 %	9.7 %		55 %	86 %	5.7 %	*	20 %
3apr13ROV	MUT					1.1 %	0.9 %		
5Apr13ROV	VERIFIN								*
6Apr13BOX	MUT					1.3 %			
6Apr13ROV	FOI				0.02 %			0.03 %	
10Apr13ROV	MUT					24 %	0.4 %		
14Apr13BOX	MUT					25 %			
ROV3	MUT						0.5 %	2.4 %	
ROV9	MUT					20 %			
Average		57 %	10 %	2 %	26 %	24 %	2 %	3 %	20 %
SD		62 %	–	1 %	23 %	27 %	3 %	3 %	–

* Chemical was found in pore water sample, but not in sediment

3.2 Findings by chemical warfare agent

3.2.1 Sulphur mustard-related chemicals

Sulphur mustard (1)

One sample was found to contain intact sulphur mustard. The concentration was well below the LOQ. The estimated concentration was 0.030 µg/kg dw. This finding was made only by VERIFIN, but in the repeated analysis on the same sample (sample preparation started from wet sediment) confirmed the analysis. Also, the identification fulfilled all used identification criteria for retention time and ion ratios (see Figure 6).

The presence of intact agent in the sediment could be interpreted as evidence of recent release of mustard from a container such an artillery shell or an aerial bomb.

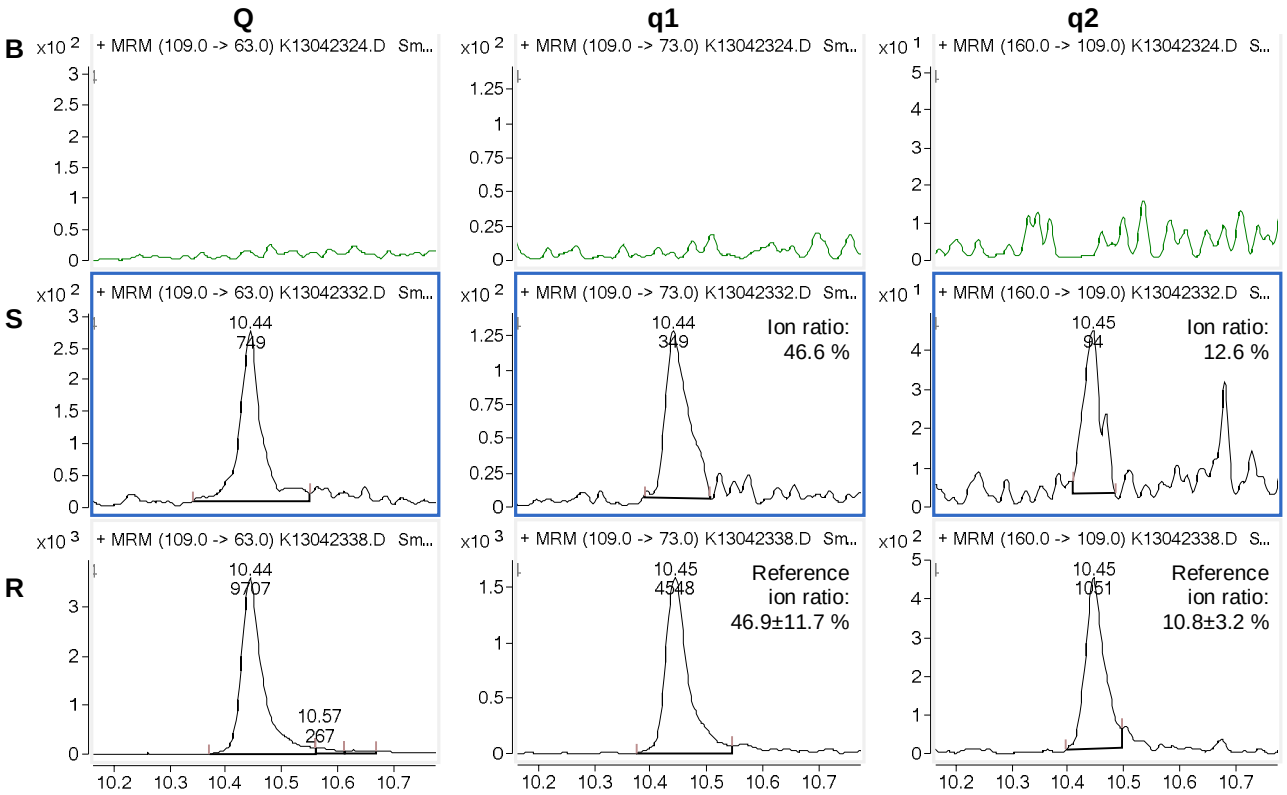

Figure 6. Detection of sulphur mustard (**1**) using GC–EI/MS/MS in sample *WH349/B13/3* (Bornholm dumpsite at an estimated conc. of 0.030 µg/kg dw. SRM chromatograms for (B) blank sediment sample, (S) sample *WH349/B13/3* and (R) reference sample (5 ppb). Used transitions: Q = quantifier, q1 and q2 = qualifiers. *[From report by VERIFIN]*

Thiodiglycol (1.1). bis(2-siloxyethyl)sulphide (1.1S), thiodiglycol sulfoxide (1.1O) and bis(2-siloxyethyl)sulfoxide (1.1OS)

Thiodiglycol and its oxidation product were analysed in the samples with both GC–MS (after silylation) and LC–MS (intact and after oxidation).

The only findings of these chemicals were made by MUT. Four individual sediment samples contained **1.1OS** (i.e. **1.1O** after silylation) at quite high concentration (200–610 µg/kg dw). Data for the finding in sample *ROV4* is shown in Figure 7. Three of these samples were received only by MUT, but one was analysed also by VERIFIN and no respective **1.1O** could be identified (LOD 8.8 µg/kg dw).

Samples from two core samples were found by MUT to contain **1.1S**. Core sample *K_1857* between contained **1.1S** between depths 10–17.5 cm and 20–25 cm. Representative data is shown in Figure 8. Also slice 22.5–25 cm of core sample *Q_7466* contained **1.1S**. It should be noted that each slice of these core samples had been divided in two aliquots. Chemical **1.1S** could not be detected in the other aliquot of the same slice.

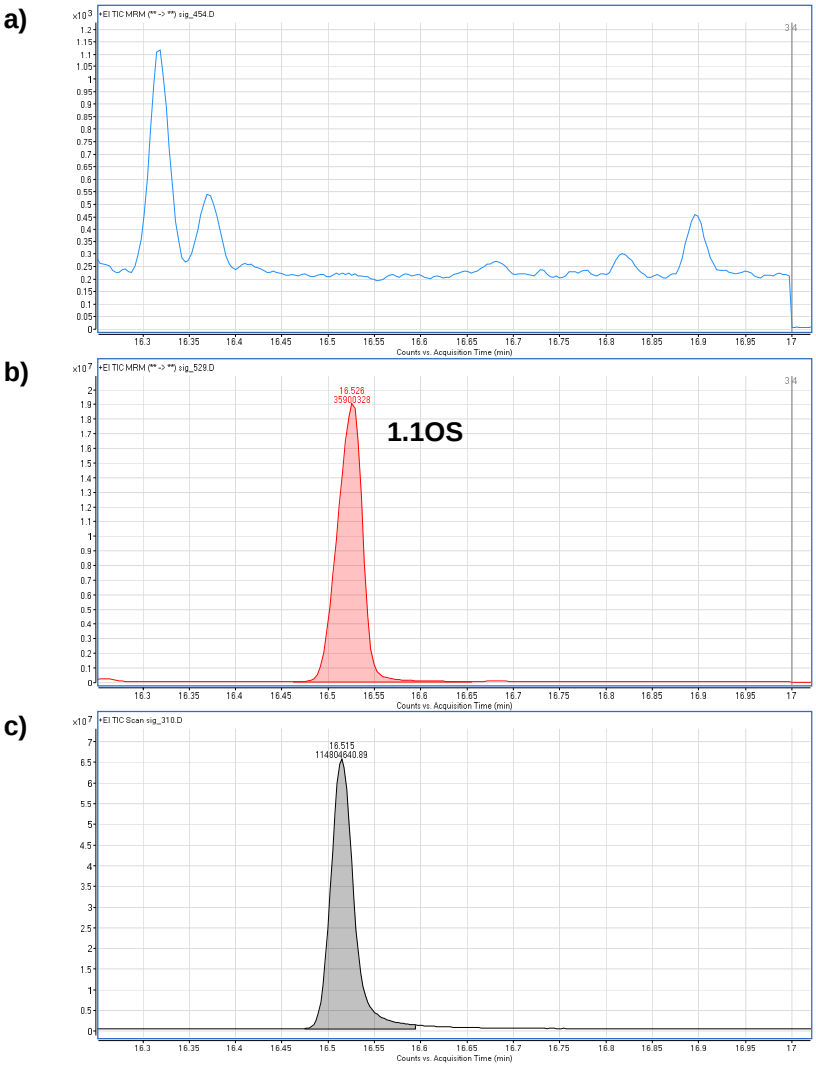

Figure 7. GC–EI/MS/MS chromatograms obtained during the analysis of:
a) blank sample,
b) sediment sample “ROV 4” and
c) thiodiglycol sulfoxide standard (**1.1OS**). *[Data by MUT]*

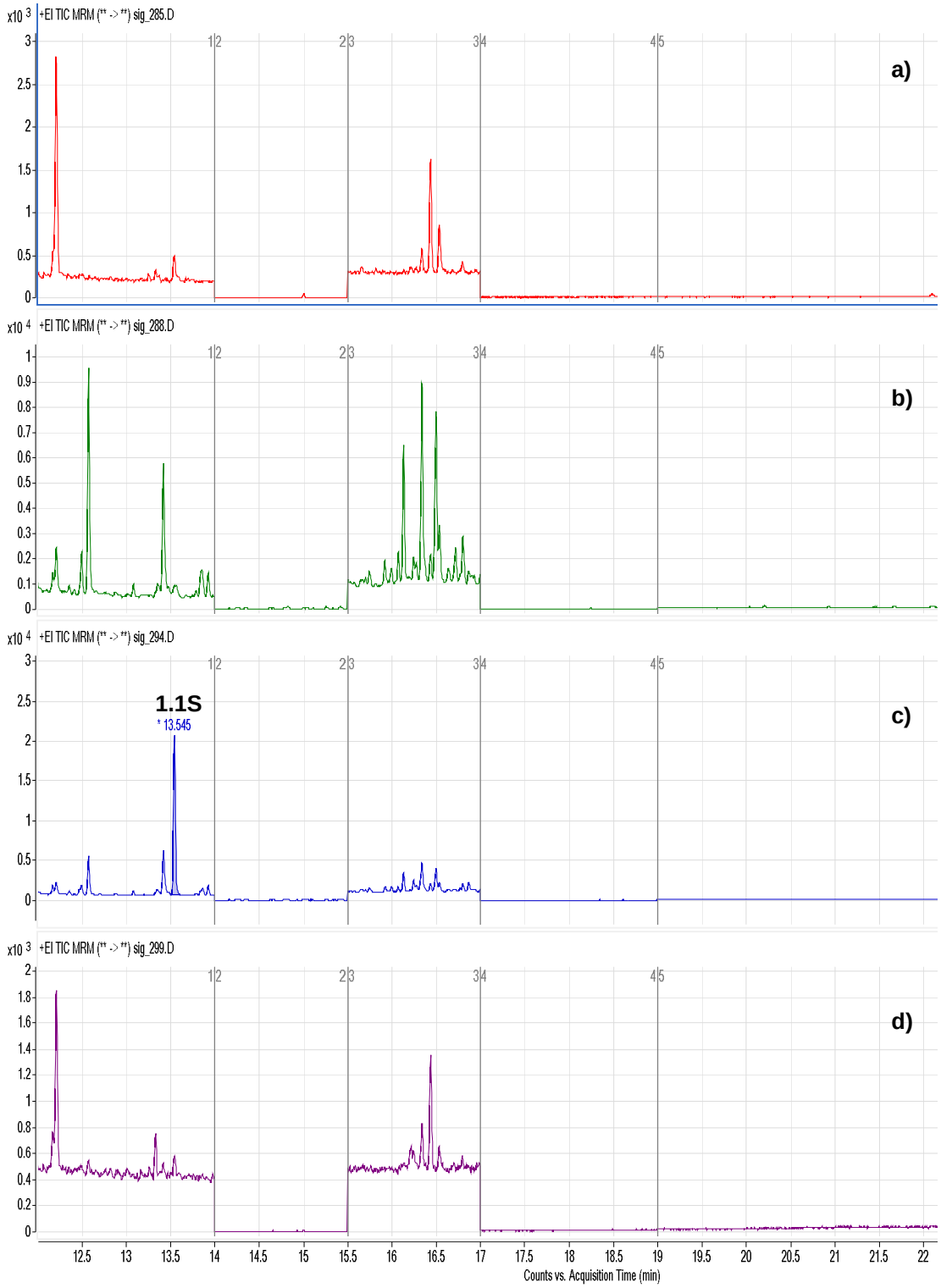

Figure 8. GC–EI/MS/MS chromatograms obtained during the analysis of:
a) acetonitrile with BSTFA (at the start of batch) ,
b) core sample *"K_1857 a) 0–5cm"*,
c) core sample *"K_1857 a) 12.5–15cm"* and
d) acetonitrile with BSTFA (at the end of batch). *[From report by MUT]*

1,4-Dithiane (1.2) and 1,4-dithiane oxide (1.2O)

Chemical **1.2** is often considered to be an important degradation product of sulphur mustard. In these analyses, 1.2 was found in six samples by both MUT and VERIFIN in concentrations from 0.52 to 25 µg/kg dw. In four cases, also other cyclic mustard degradation products (**1.4** and/or **1.5**) could be detected in the same samples.

FOI performed the analysis of **1.2** using GC–MS while MUT and VERIFIN used GC–MS/MS. The LOQ value for GC–MS was14 µg/kg dw and there were considerable interferences from the background. GC–MS/MS had virtually no background and the LOQs were much lower (MUT 0.31 µg/kg dw; VERIFIN 0.45 µg/kg dw).

All laboratories had problems in getting a good standard for **1.2O**. VERIFIN purchased a commercial standard from Aldrich while FOI and MUT synthesized the chemical. The commercial chemical was not pure and the synthesized products were not of satisfactory purity. It was however noted by FOI during the analysis that **1.2** disappeared from the sample. This may have happened due to spontaneous oxidation to **1.2O**. The same effect was noted by VERIFIN during the preparation of the samples for inter-calibration study. No findings were made in the samples for **1.2O**. In later tests, FOI noted that this type of oxidation occurred when dithiane was spiked in freeze-dried sediment, but not when spiked in wet sediment.

1,4-Oxathiane (1.3), 1,4,5-oxadithiepane (1.4) and 1,2,5-trithiepane (1.5) and 1,7-dioxa-4,10-dithiacyclododecane (1.6)

These cyclic degradation products are possibly formed also from other sulphur mustard-type chemicals that the sulphur mustard (**1**) itself. In technical mustard, there can be some by products present, e.g. sesquimustard [bis(1,2-chloroethylthio)ethane]. In mustard munitions, various reactions are also transforming mustard into more complex molecules eventually leading to polymerisation of the material. Some of these transformation products could be the source of chemicals **1.3** through **1.6**.

All the cyclic degradation products were analysed in the samples using GC–MS (LOQs 3–6 µg/kg dw) or GC–MS/MS (LOQs 0.45–13 µg/kg dw). In the GC–MS method, the identification of **1.3** may have suffered from interfering peaks. Only real problem in the analysis of these chemicals were caused by **1.6**. FOI reported that it was not detectable after spiking in sediment extracts and MUT had a slightly higher LOQ (6.3 µg/kg) for it. Reason for these findings could have been either instability of the compound or matrix effects.

Chemical **1.3** was detected by MUT only in three samples (concentration range 0.3–122 µg/kg dw).

Chemical **1.4** was detected in 16 samples. It should be noted that identifications of this chemical were divided between laboratories quite imbalanced: FOI in four samples (4.2–14 µg/kg dw), MUT in 12 samples (9.8–40 µg/kg dw) and VERIFIN in one sample (2.8 µg/kg dw). Only in one case the chemical was identified in a divided sample by two laboratories. No clear explanation for this imbalance between numbers of findings was found.

Chemical **1.5** was detected in 16 samples. The findings of this chemical were more balanced than was the case for **1.4**: FOI in four samples (5–35 µg/kg dw), MUT in eight samples (0.5–9.2 µg/kg dw) and VERIFIN in five samples (0.60–28 µg/kg dw).

It should be noted that both **1.4** and **1.5** were identified in seven samples. In one case **1.3** and **1.4** was found in the same sample.

Chemical **1.6** was not detected by any of the laboratories.

Thiodiglycolic acid (1.7) and bis(trimethylsilyl) 2,2'-thiodiacetate (1.7S)

Chemical **1.7** and its trimethylsilyl derivative **1.7S** (required for GC analysis) was selected to the list of target chemicals as there were indications that it would be a bacterial degradation product of thiodiglycol **1.1** in the sediment.

FOI and MUT analysed the chemical **1.7S** using GC–MS (LOQ 3 µg/kg dw) and GC–MS/MS (LOQ 6.3 µg/kg dw), respectively. FOI and VERIFIN analysed intact **1.7** using LC–MS/MS, but both reported problems with this chemical. Neither laboratory could find it after spiking to sediment extracts with the applied analysis methods. FOI spiked the chemical in solvent to be used as a reference and VERIFIN only screened for the presence of the chemical.

Only MUT could identify **1.7S** in two samples. The findings were at quite high level (530–550 µg/kg dw). FOI analysed the divided fractions of the same two samples, but could not detect anything in the samples. No explanation for this could be found.

Summary of mustard-related findings

Three types of degradation products were detected for sulphur mustard (**1**): hydrolysis products (thiodiglycol [**1.1**] and thiodiglycol sulphoxide [1.1O]), cyclic degradation products (**1.2**–**1.5**) and bacterial degradation product thiodiglycolic acid (**1.7**). These chemicals and the summary of findings are presented in Figure 9 below.

In general, the transfer of mustard-related chemicals into pore water samples seems quite low as illustrated by Figure 10.

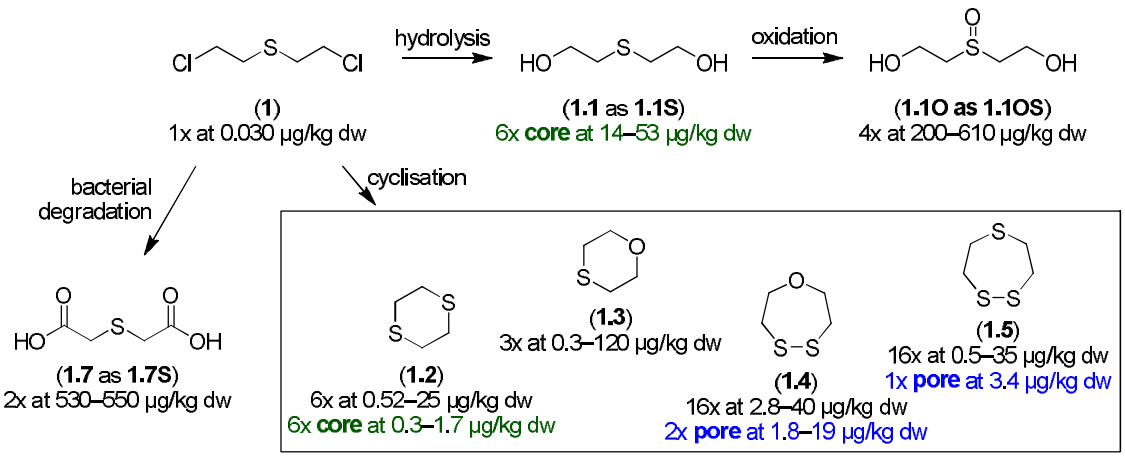

Figure 9. Summary of degradation of sulphur mustard and findings for the target chemicals. The number of findings and the concentration range is shown for each chemicals.

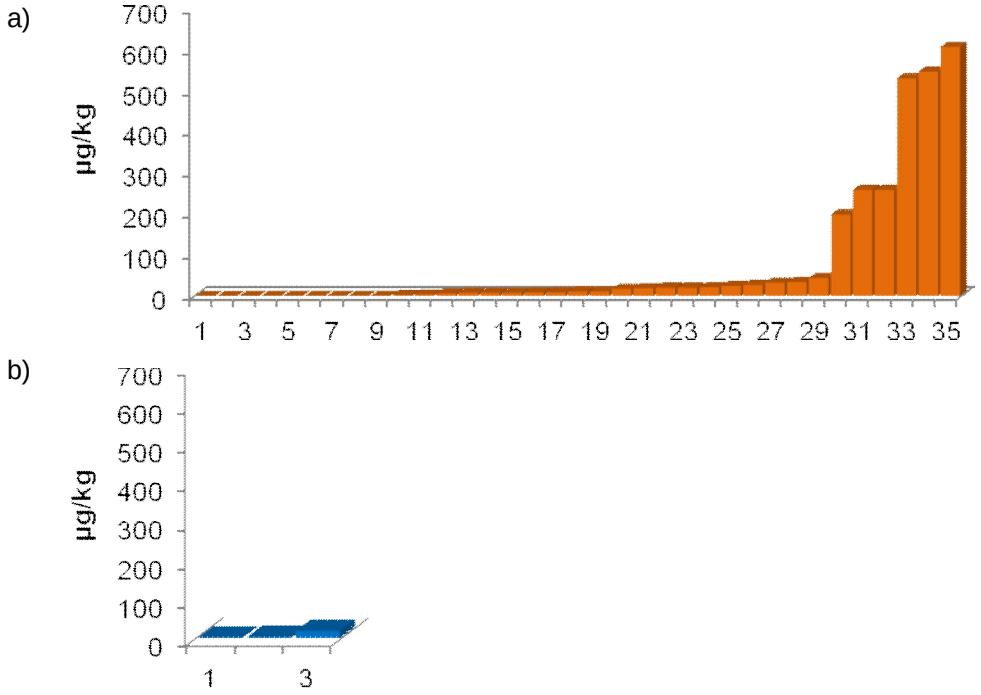

Figure 10. Concentration distrubution of positive sulphur mustard-related findings in: a) sediment portions (25 positive samples) and b) pore water portions (3 positive samples).

It seems that these chemicals are found in different samples at different concentrations so that no clear marker chemicals can be picked up. Only mustard-related target chemical that could be left out from a future list of target chemicals could be 1,7-dioxa-4,10-dithiacyclodeodecane (**1.6**), which was not found in any of the samples.

Although, several samples contained degradation products for sulphur mustard (**1**), there were clearly fewer findings at lower concentrations than for arsenic-containing chemicals. Still, mustard is the most dumped agent in Baltic Sea. Several hypotheses for the low amount of mustard-findings can be presented:

- Mustard is in an form not detected in the current analysis. This would mean that there could be still identified abundant degradation products in sediment. The degradation of mustard in sediment should be studied in laboratory studies.
- Mustard is not released from munitions/containers and subsequently spreading to the sediment. This could be due to formation of poorly soluble or totally insoluble lumps captured by fishermen.
- Mustard is removed from the sediment surrounding the dumped munitions and containers. This could occur e.g. by hydrolysis and subsequent dissolution in sea water and dilution beyond detection. Also, it has been speculated that bacteria could consume thiodiglycol in the sediment.
- Mustard does not get bound to the sediment after it has been released from munitions or containers. One way this could occur is that mustard is actually hydrolysed rather than bound to the sediment.

It should be remembered that mustard is not very soluble in water and in the conditions at the sea bottom mustard is solid material as the water temperature is well below the melting point of mustard.

3.2.2 Arsenic-containing chemicals

Adamsite-related: 5,10-Dihydrophenoarsazin-10-ol 10-oxide (2O) and 10-(propylthio)-5,10-dihydrophenarsazinine (2T)

Adamsite-related degradation products were analysed from oxidized subsample (as **2O**) using LC–MS/MS by FOI and VERIFIN. FOI also analysed the propane-1-thiol derivative (**2T**). FOI reported problems with the derivatisation in both calibration and real samples (e.g. chemical **2T** was not detected after spiking in sediment extracts). MUT reported that the derivatisation did not occur. VERIFIN did not test the derivatisation since it had previously noticed similar tendencies and FOI and MUT. The LOQ values for **2O** were 1 and 4.4 µg/kg dw by FOI and VERIFIN, respectively.

The oxidised degradation product was identified in 13 samples (in eight samples by FOI and in seven by VERIFIN). In two cases, both laboratories identified the chemical in sediment samples divided between the laboratories. The found concentrations varied from 2.5 to 1400 µg/kg dw.

It seems that, for Adamsite-related degradation products, the oxidation is a much more reliable and robust method than the derivation with propane-1-thiol.

The transfer of Adamsite-related chemicals into pore water as shown in Figure 11 is low.

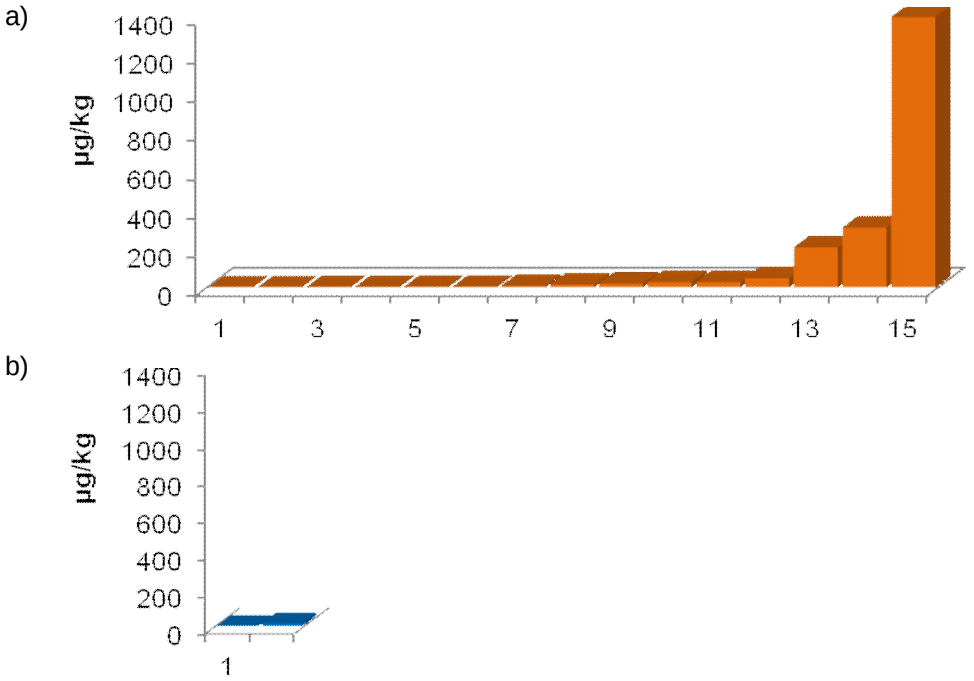

Figure 11. Concentration distribution of positive Adamsite-related findings in: a) sediment portions (15 positive samples) and b) pore water portions (2 positive samples).

Clark-related: Diphenylarsinic acid (3O) and diphenylpropylthioarsine (3T)

Clark-related degradation products were analysed by all three laboratories: from oxidized subsample (as **3O**) using LC–MS/MS by FOI and VERIFIN (LOQs 2 and 8.7 µg/kg dw, respectively) and as propane-1-thiol derivative (**3T**) by either GC–MS (FOI; LOQ 15 µg/kg dw) or GC–MS/MS (MUT and VERIFIN; LOQs 13 and 2.1 µg/kg dw).

The Clark-related degradation products were the most widely spread chemicals in the sediment samples as **3O** and/or **3T** were found in 25 samples (**3O** in 16 and **3T** 17 in samples). Both chemicals were found in five samples. In four cases two laboratories found **3O**/**3T** in a divided

sample. The concentrations for **3O** were in range 1.6–1700 µg/kg dw and for **3T** in range 13–490 µg/kg dw.

In seems that to obtain a full perspective of contamination by Clark-related chemicals, it is necessary to perform both GC and LC-based analyses using both derivatisation with propane-1-thiol and oxidation. The results of the two methods are quite variable probably due to several factors: e.g. inhomogeneity of the sediment, the actual form of the degradation products in the sediment, different extraction efficiencies of different solvents and different reactivity of the derivatisation/oxidation reagents.

Based on pore water sample analysis, in some cases quite high amounts on degradation products of Clark-related chemicals can be transferred into pore water (shown in Figure 12).

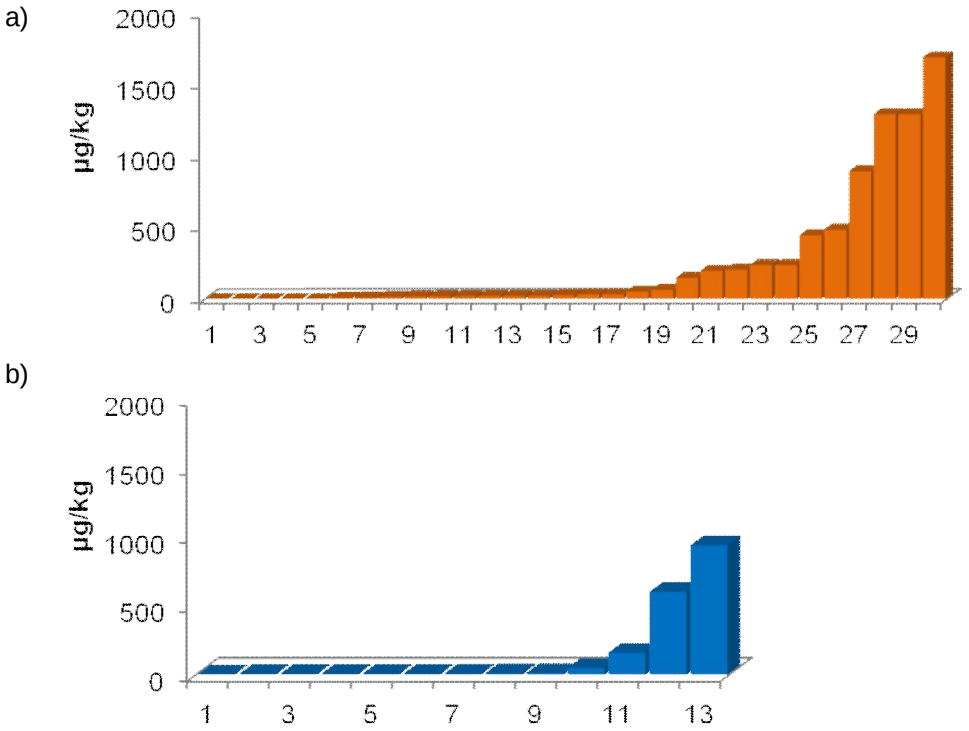

Figure 12. Concentration distribution of positive Clark-related findings in: a) sediment portions (30 positive samples) and b) pore water portions (13 positive samples).

Triphenylarsine (4) and triphenylarsine oxide (4O)

The intact chemical **4** was analysed by GC–MS(/MS) method by all laboratories (LOQs: FOI 6 µg/kg dw, MUT 1.6 µg/kg dw, VERIFIN 2.3 µg/kg dw). The oxidised chemical (**4O**) was analysed using GC/MS/MS by MUT (LOQ 3.1 µg/kg dw) and using LC–MS/MS by FOI and VERIFIN (LOQs 3 [in solvent] and 4.4 µg/kg dw). The method used by MUT for **4O** is based on reduction of **4O** back to **4** in the injection to GC. FOI reported that **4O** was not detected after spiking to sediment extracts and therefore it was spiked in solvent to be used as a reference.

Chemicals **4** and/or **4O** were detected in 18 samples (**4** in 16 samples and **4O** in eight samples). Both chemicals were detected in five samples. In one case two laboratories found **4**/**4O** in a divided sample. The concentration ranges were 0.7–1200 µg/kg dw for **4** and 35–590 µg/kg dw for **4O**.

Low portion of triphenylarsine and its oxidised form seem to be transferring into pore water (see Figure 13).

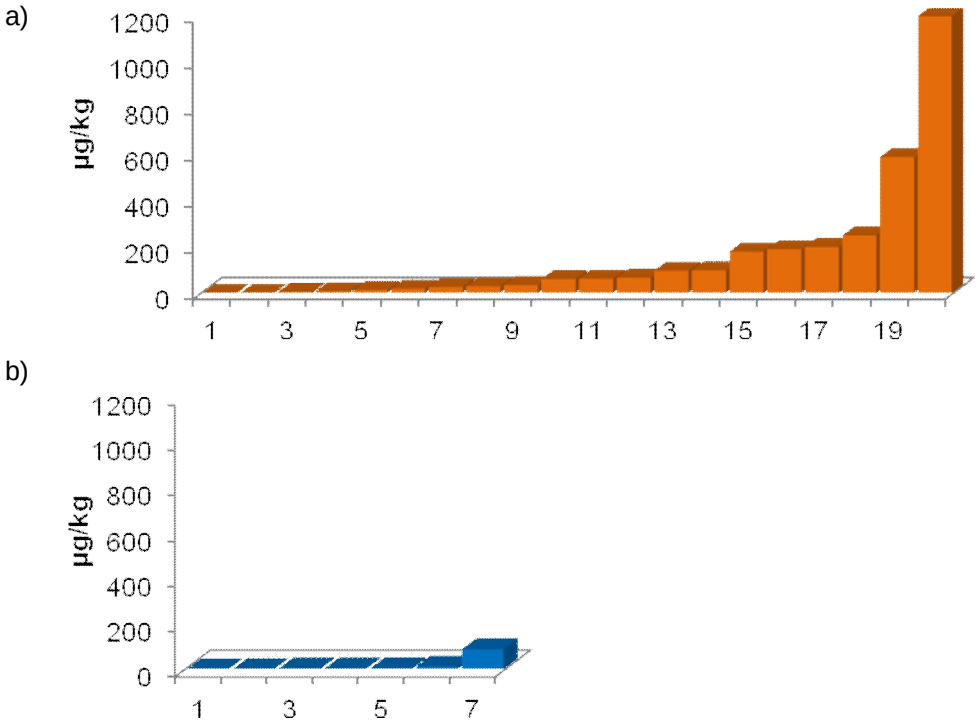

Figure 13. Concentration distrubution of positive triphenyl arsine-related findings in: a) sediment portions (20 positive samples) and b) pore water portions (7 positive samples).

As with Clark-related chemicals, both analysis of **4** and **4O** are necessary to obtain complete picture of the contamination.

The GC peak shape of **4** becomes quite wide in the sediment extracts (Figure 14 and Figure 15, left), while it is much better in pure solvent. The width of the peaks (at half-height) are in the range of 0.3 min while typical width of a GC peak is in the range of 0.1 min. FOI noticed that in silylated extract (although the chemical itself does not silylate) the peak becomes significantly better (Figure 15, right)

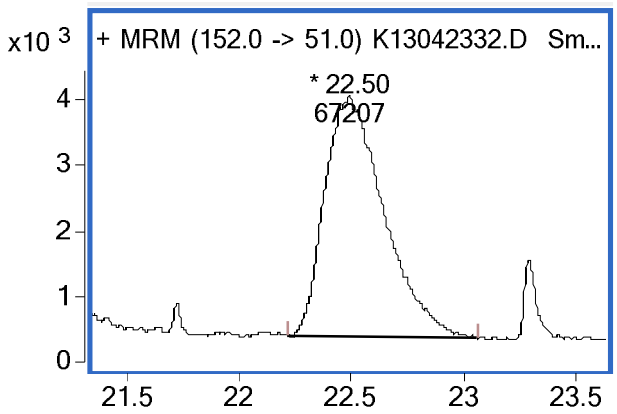

Figure 14. The quantifier transition for chemical **4** in sample *WH349/B13/3* (Bornholm dumpsite; conc. 8.0 µg/kg dw) using GC–MS/MS. *[Modified from report by VERIFIN]*

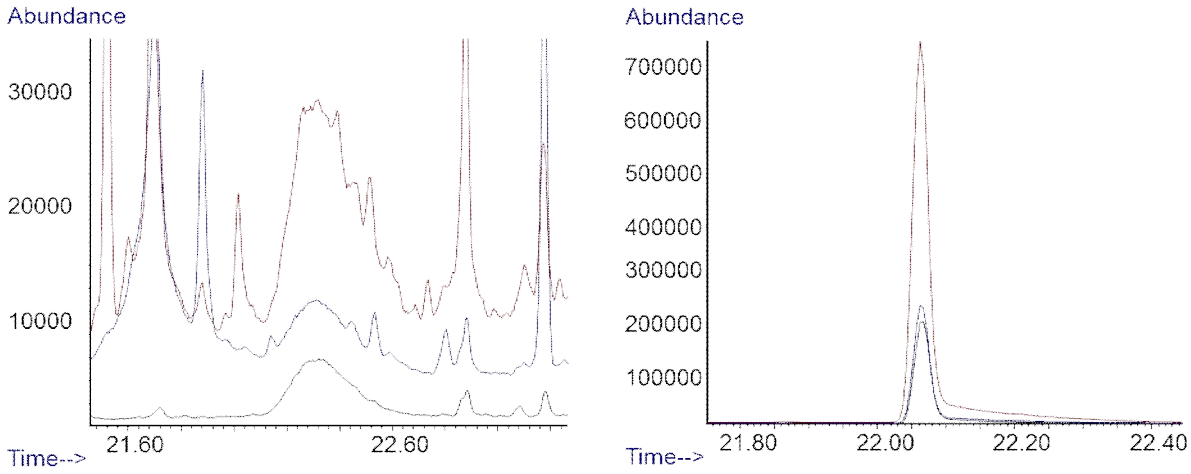

Figure 15. Left) GC-MS chromatogram (m/z 152, 227, and 306) of TPA in dichloromethane extract of sample 6Apr13ROV. Right) GC-MS chromatogram of TPA in silylated combined acetonitrile-dichloromethane extract of sample 6Apr13ROV (CHS074). *[from report by FOI]*

Phenyldichloroarsine-related: Phenylarsonic acid (5O) and dipropyl phenylarsono-dithioite (5T)

The oxidized degradation product (**5O**) was analysed by only FOI using LC–MS/MS (LOQ 1 µg/kg dw). VERIFIN screened **5O** in pore water samples. The propane-1-thiol derivative (**5T**) was analysed by all laboratories by either GC–MS (LOQ 18 µg/kg dw) or GC–MS/MS (LOQs 3.1 and 2.1 µg/kg dw).

Chemical **5O** and/or **5T** was identified in 18 samples. **5O** was identified in three samples at concentrations 19–1300 µg/kg dw and **5T** in 16 samples at concentrations 3.2–570 µg/kg dw.

The findings for 5T are slightly imbalance between laboratories: FOI made two findings (note the higher LOQ), MUT made one finding below FOI's LOQ and eight above and VERIFIN made three findings below FOI's LOQ and one above. Five of the MUT's higher concentration findings were made in Gdańsk Deep and Słupsk Furrow.

Only low concentrations of phenyldichloroarsine-related degradation products were detected in pore water.

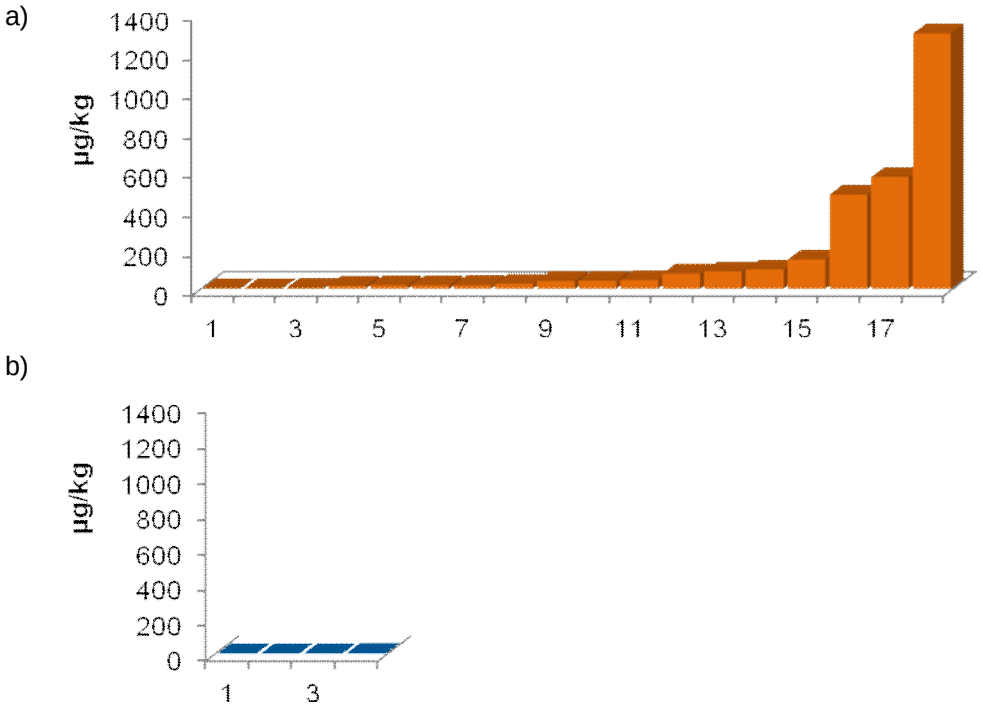

Figure 16. Concentration distrubution of positive Phenyldichloroarsine-related findings in: a) sediment portions (18 positive samples) and b) pore water portions (4 positive samples).

Lewisite I-related: 2-Chlorovinylarsonic acid (7O) and dipropyl (2-chlorovinyl)arsono-dithioite (7T)

The oxidized degradation product (**7O**) was analysed by only FOI using LC–MS/MS (LOQ 2 µg/kg dw in solvent). The chemical could not be detected after spiking in sediment extracts. FOI also reported that analysis of **7O** was problematic probably due to suppression effects as the retention time in reverse-phase liquid chromatography was low. VERIFIN did not analyse for **7O** as the method performance was not satisfactory. The propane-1-thiol derivative (**7T**) was analysed by all laboratories by either GC–MS (LOQ 32 µg/kg dw) or GC–MS/MS (LOQs 3.1 and 2.1 µg/kg dw). The higher LOQ for FOI was due to interfering peaks.

No findings were made for either **7O** or **7T**.

Lewisite II-related: Bis(2-chlorovinyl)arsinic acid (8O) and bis(2-chlorovinyl) propylthio-arsine (8T)

The oxidized degradation product (**8O**) was analysed by FOI and VERIFIN using LC–MS/MS (LOQs 8 and 2.2 µg/kg dw). The propane-1-thiol derivative (**8T**) was analysed by all laboratories by either GC–MS (LOQ 7 µg/kg dw) or GC–MS/MS (LOQs 1.6 and 2.1 µg/kg dw).

It is noteworthy that degradation products for Lewisite II are much easier to analyse than those for Lewisite I.

No findings were made for either **8O** or **8T**.

Summary of arsenic-containing chemicals

Three of the arsenic-containing target chemicals required derivatisation and/or oxidation for analysis. For Adamsite (**2**), the oxidation is a much better alternative than derivatisation with propanethiol. Laboratories reported problems in propane-1-thiol derivatisation. For Clark I (**3**), both derivatisation and oxidation worked well and similar amounts of findings were made using both methods. For phenyldichloroarsine (**5**), derivatisation gave more findings, but still the highest concentration was found using oxidation. Oxidation is the only option for triphenylarsine (**4**), but also the intact chemical can be analysed. Both methods worked well, but more findings were made for the intact chemical. Figure 17 summarises the findings for arsenic-containing chemicals.

With Clark I, triphenylarsine and phenyldichloroarsine it can be seen that the different analysis/sample preparation methods give different results. Some samples are found positive with one and some with the other. Naturally, in some cases both provide positive results. Based on this finding, it can be stated that both methods are required to cover all target chemicals. Clearly, the samples are different from each other and different sample preparation methods as well as derivatisation reactions are required to catch all possible degradation products of the arsenic-containing target chemicals.

It also seems that Clark I has the tendency of transferring from sediment into pore water better than the other arsenic-containing target chemicals.

3.2.3 Tear gas-related chemical

α-Chloroacetophenone (6)

Chemical **6** was analysed by all laboratories as intact using GC–MS (FOI; LOQ 49 µg/kg dw) or GC–MS/MS (MUT and VERIFIN; LOQs 3.1 and 0.45 µg/kg dw). FOI reported that the rather high LOQ was due to interfering peaks.

Two findings of **6** were made by MUT only at relatively low level (7.0–7.5 µg/kg dw). One of the samples was analysed only by MUT. In the other sample divided also to VERIFIN no chemical **6** could be identified.

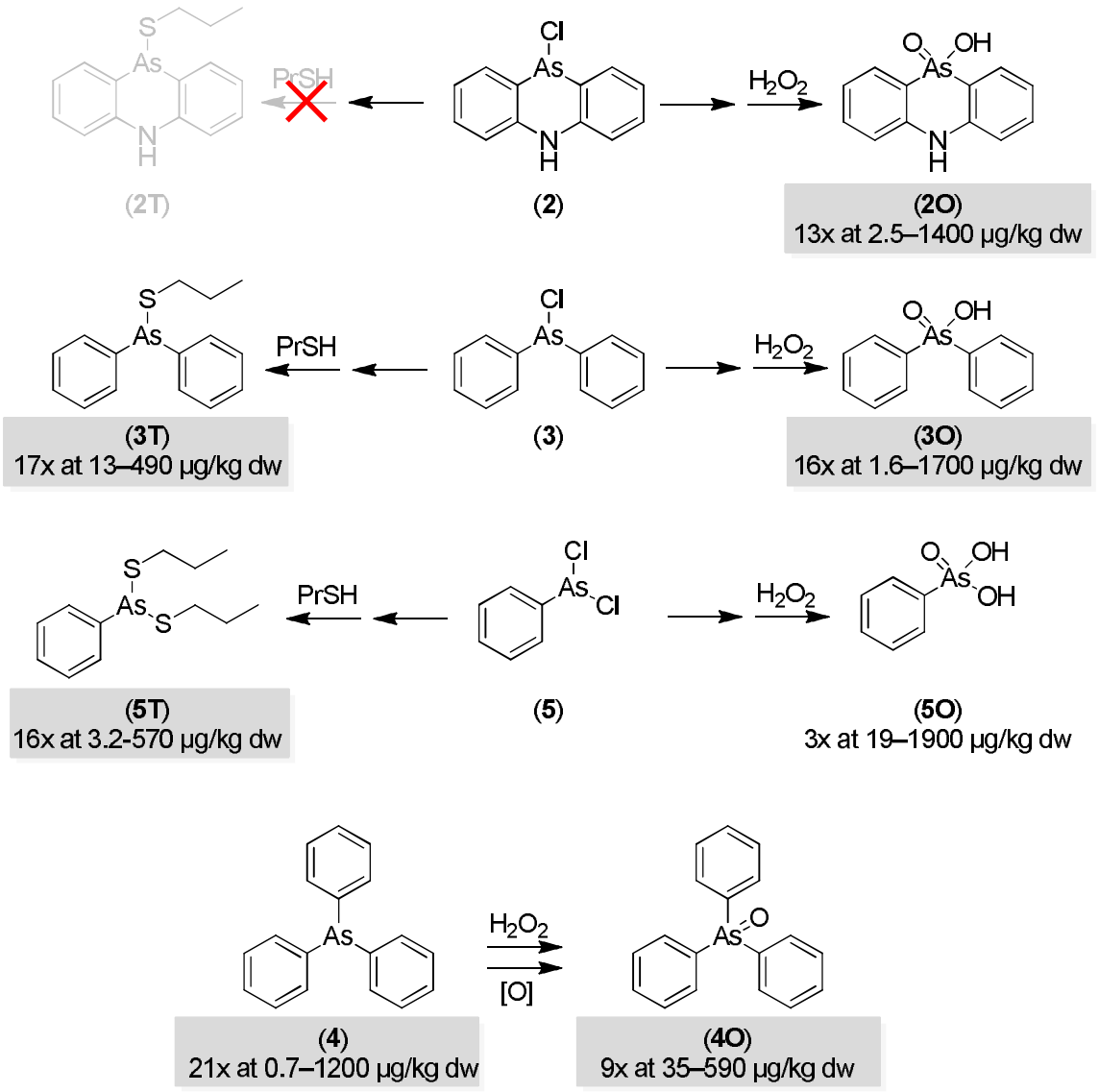

Figure 17. Summary of findings of derivatisation products of arsenic-containing chemicals. The number of findings and the concentration range is shown for each chemicals.

3.3 CWA findings by area

The main sampling site for the CHEMSEA project was Gotland Deep, with 84 samples. The other two sites with more than 20 samples were Bornholm and Gdańsk Deeps. The highest relative number of findings was made in Bornholm area, which is known to be the major dumpsite in the Baltic. The locations of good sampling sites in Bornholm were based of data from the MERCW project. The other areas have not been studied earlier. Figure 18 summarises the number of positive and negative findings in the sampling areas. Following subchapters 3.3.1 through 3.3.5 discuss the findings in each sampling area.

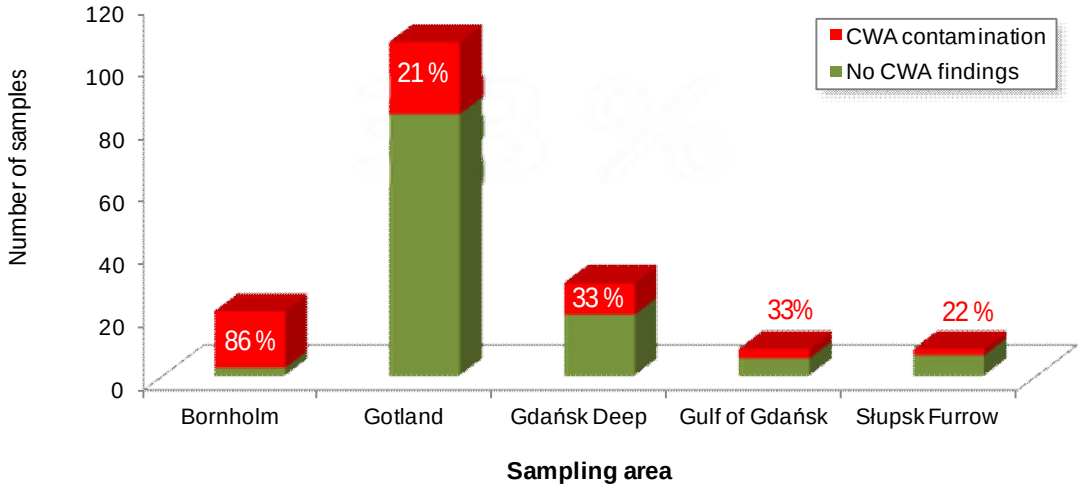

Figure 18. Amount of CWA contaminated samples in different sampling areas.

3.3.1 Dumpsite at Bornholm Deep

In total, 18 of the 21 samples taken at Bornholm Deep were positive. This means that 86 % of the samples were contaminated. These findings are in line with previous findings by MERCW Project. **The amount of contamination is remarkable.** The findings are shown on a map in Figure 19.

Most of the identified target chemicals were found also in Bornholm. The only chemical not detected there were **1.1OS** (silylated **1.1O**), **1.3** and **1.7S** (silylated **1.7**), which are all degradation products of sulphur mustard.

The highest concentrations of arsenic-containing chemicals were made in Bornholm. Also intact sulphur mustard was detected in one sample. Most interesting findings were four samples were two laboratories found more chemicals: *WH349/B13/3*, *3Apr13BOX*, *6Apr13BOX* and *7Apr13BOX*. The findings are illustrated graphically from Figure 20 to Figure 23. Findings are similar but there are large differences between findings. This is most probably due to inhomogeneity of the sediment samples.

It seems that the chemicals are very unevenly distributed in the sediment samples at the molecular level. They may be forming some kind of clusters, which are not easy to homogenize.

The findings made in Bornholm Deep match well with the results obtained by the MERCW Project. The finding of intact sulphur mustard was the first time in the area. Also, addition of the cyclic mustard degradation products to the list of target chemicals proved to be a success.

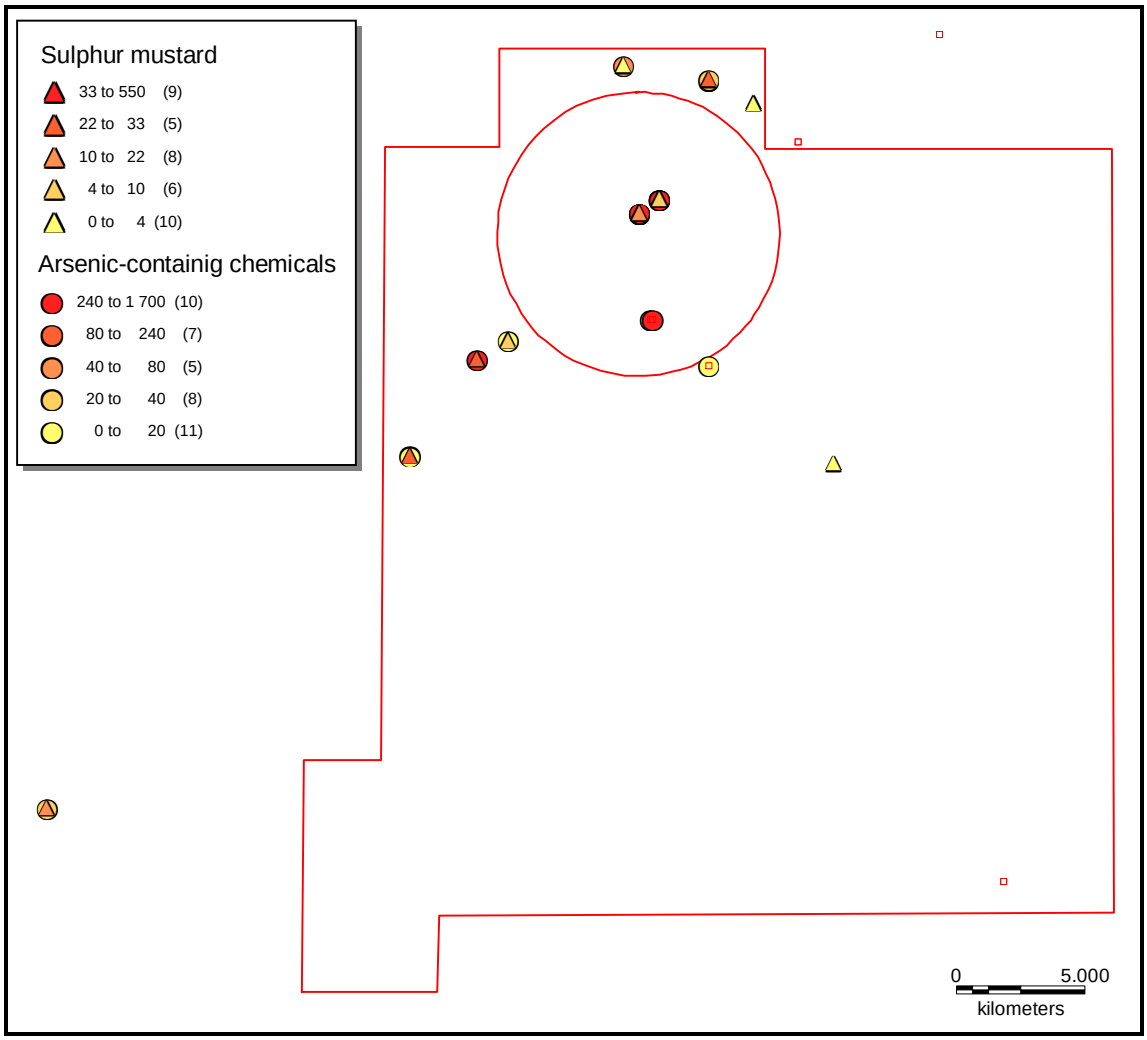

Figure 19. Distribution of positive findings in sediment samples in Bornholm Deep area. *(dumpsite border shown in red).*

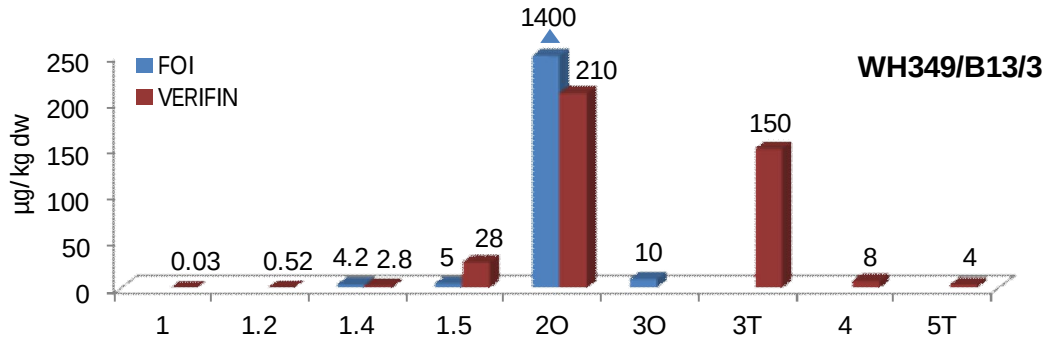

Figure 20. Findings by FOI and VERIFIN in sample *WH349/B13/3* taken in Bornholm Dumpsite.

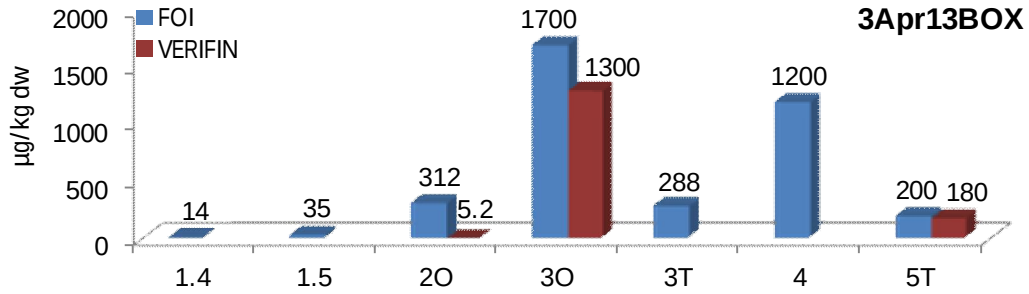

Figure 21. Findings by FOI and VERIFIN in sample *3Apr13BOX* taken in Bornholm Dumpsite.

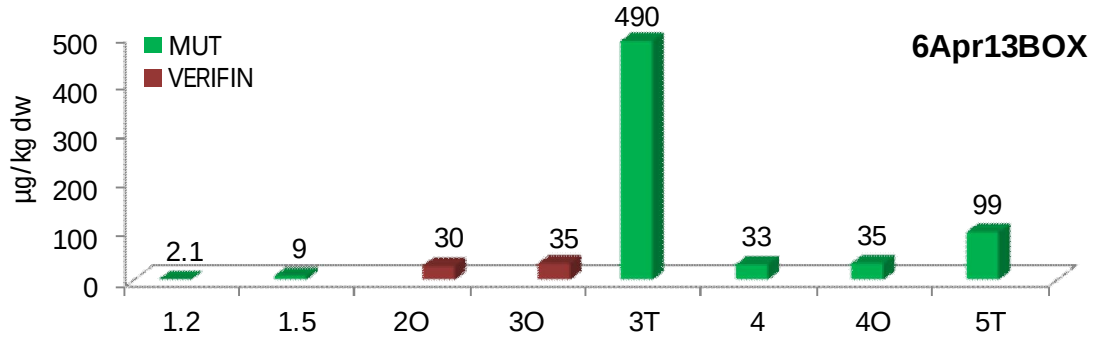

Figure 22. Findings by MUT and VERIFIN in sample *6Apr13BOX* taken in Bornholm Dumpsite.

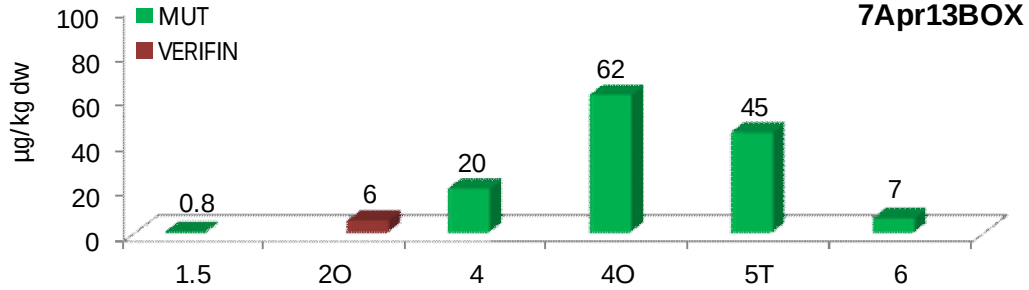

Figure 23. Findings by MUT and VERIFIN in sample *7Apr13BOX* taken in Bornholm Dumpsite.

3.3.2 Dumpsite at Gotland Deep

Gotland Deep was the most sampled area in the CHEMSEA Project. In total, 23 of the 84 samples taken in the area were positive. Findings are shown on a map in Figure 24.

It should be noted that samples were taken only in the Swedish and Lithuanian exclusive economic zones (EEZ). Only one of the ten samples in Lithuanian EEZ was positive. Also, the average arsenic concentration in the Lithuanian EEZ was lower (6.1 mg/kg) than in the Swedish EEZ (13.8 mg/kg) (see discussion in chapter 3.4.2).

Only cyclic degradation products were found in the sediment for sulphur mustard (**1.2**, **1.3**, **1.4** and **1.5**). Most of the target chemicals related to Adamsite (**2O**), Clark (**3O** and **3T**), triphenyl arsine (**4**; not **4O**) and PDCA (**5O** and **5T**) were identified. Maximum concentrations of all found chemicals were smaller than those in the samples taken from Bornholm Deep.

These findings are in line with the known amounts of dumped munitions when compared to the Bornholm Deep, which was the major dumpsite in the Baltic Sea.

It is interesting to note that the findings concentrate to the south-western quadrant of the dumpsite. This is kind of expected as this area is closest to Germany. The vessels performing the dumping runs have probably dumped the munitions as soon as they have been within the designated dumping area.

In this area, positive sampling stations are surrounded by near-by sampling stations with negative findings. This seems to be typical to the dumping sites in Baltic as similar observations were made already during the earlier MERCW project. This finding in combination with the molecular level inhomogeneity observed in the sediment samples lead to a conclusion that it is not possible to get two totally identical samples during the sediment sampling as the contamination is unevenly distributed from munitions to molecular level in a fractal pattern.

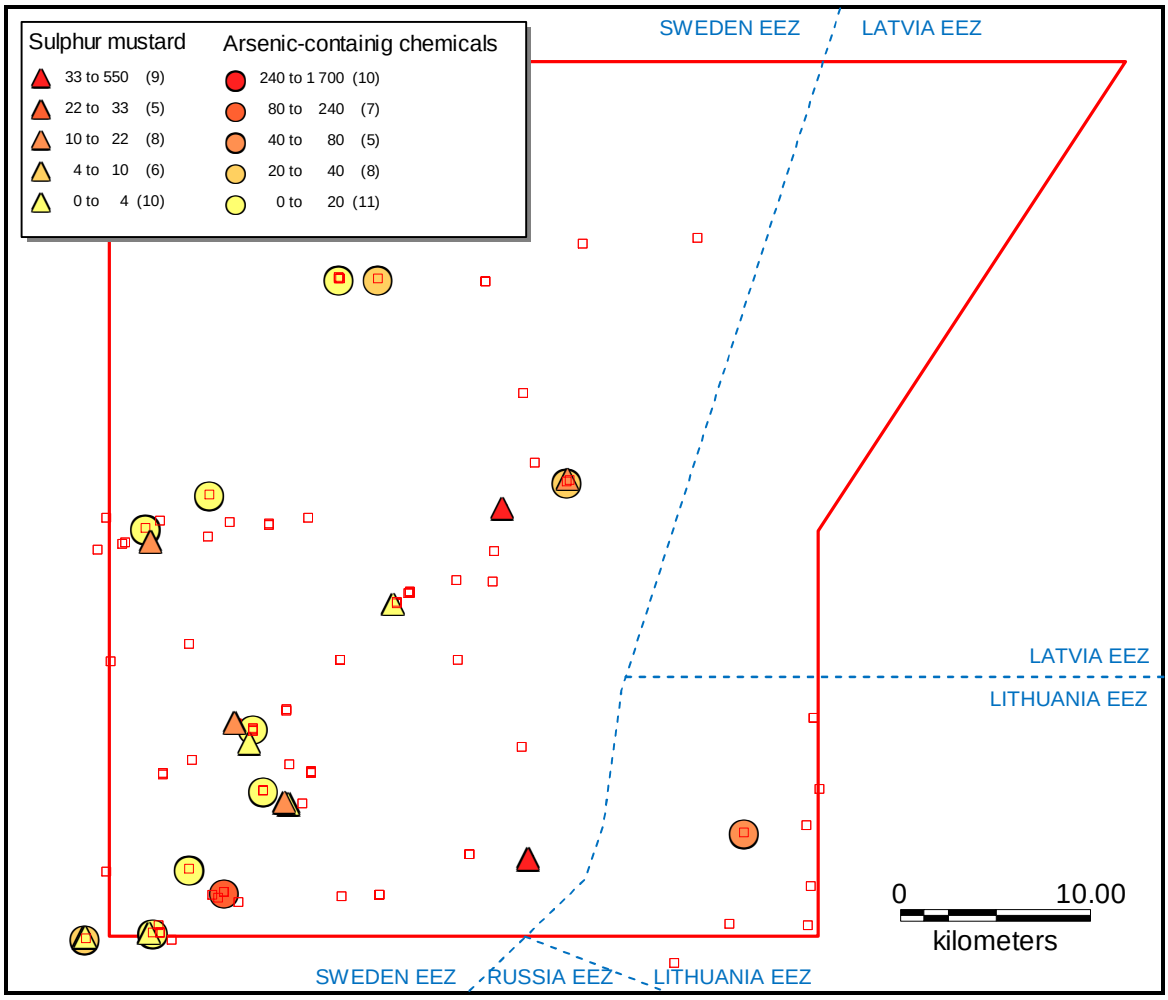

Figure 24. Distribution of positive findings in sediment samples in Gotland Deep area. *Dumpsite border shown in red. Exclusive economic zone (EEZ) borders in dashed blue line.*

3.3.3 Suspected dumpsite at Gdańsk Deep

The Gdańsk Deep was selected as a sampling site as there have been indications that munitions may have been dumped in the area. These dumping would have taken place later than the major dumping campaign at Gotland and Bornholm Deeps by the Allies after the World War II.

The total of 30 samples was taken at Gdańsk Deep and ten of the samples (33 %) were found positive. Figure 25 presents the findings at Gdańsk Deep as well as in the neighbouring areas.

Two types of degradation products were found in the area: sulphur mustard-related and those present in arsine oil including Clark I. Two samples contained both types of degradation products.

Mustard-related degradation products include thiodiglycol sulphoxide (**1.1O** analysed as silylated **1.1OS**) and several cyclic chemicals – 1,4-dithiane (**1.2**), 1,4,5-oxadithiepane (**1.4**) and 1,2,5-trithiepane (**1.5**). Two of the samples were found to contain high concentration (500–550 µg/kg dw) of thiodiglycolic acid (**1.7**), which is suspected to be a bacterial degradation product for thiodiglycol (**1.1**). This could mean that mustard (**1**) has been hydrolysed into **1.1** and then transformed by bacteria into **1.7**. *It would be interesting to study the bacterial flora of the Gdańsk Deep as **1.7** has not been found at any other studied area.*

The arsenic-containing chemicals include degradation products for Clark I (as **3T**), triphenylarsine [both intact (**4**) and oxidised (**4O**)] and phenyldichloroarsine (as **5T**).

These findings, which prove the use of Gdańsk Deep as a dumpsite, point to dumping of mustard-containing munitions. The presence of arsine oil components could be explained by presence of WWII-type German winter-grade mustard munitions. These were filled with a mixture sulphur mustard and arsine oil, so-called "Winterlost".

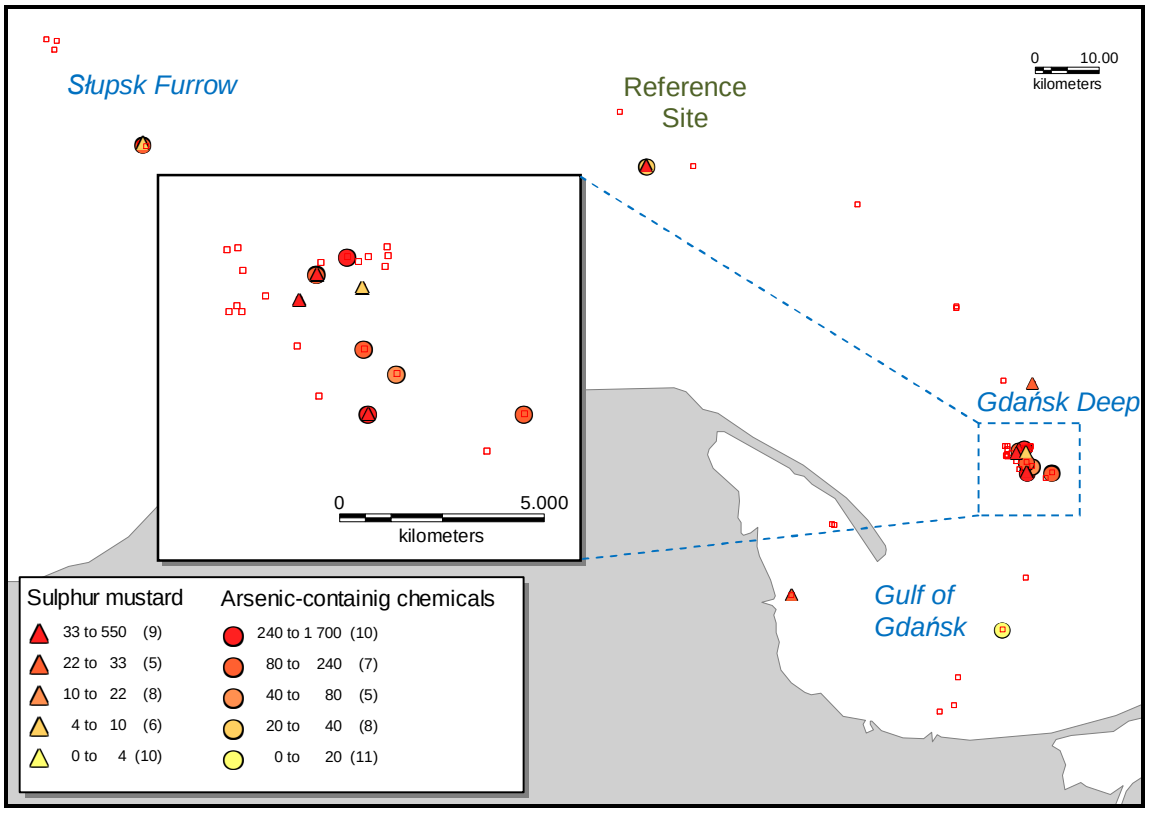

Figure 25. Distribution of positive findings in sediment samples in areas of Gdańsk Deep, Gulf of Gdańsk and Słupsk Furrow.

3.3.4 Gulf of Gdańsk

Three of the samples in Gulf of Gdańsk were found containing CWAs. These are presented in Figure 25 above.

Two samples taken ca. 3 km out from the Gdynia harbour contained degradation products for sulphur mustard: sample *1mar12* contained 25 µg/kg 1,4-dithiane (**1.2**) and *3SEP12* contained 260 µg/kg thiodiglycol sulphoxide (**1.1O** analysed as silylated **1.1OS**).

One sample in the middle of the Gulf of Gdańsk (sample *5SEP12*, ca. 25 km NE from Gdańsk) was found to contain low concentration (0.7 µg/kg) triphenylarsine (**4**), which is one of the arsine oil components.

As samples in the Gdańsk Deep contain similar chemicals as found in Gulf of Gdańsk, it is possible that these findings are related to the dumping operations at the Gdańsk Deep. It is also possible that the findings (especially the mustard-related) could be related to other events such as fishermen throwing munitions off the vessels before entering the harbour.

3.3.5 **Sł**upsk Furrow

Only eight samples were taken in the Słupsk Furrow area. Two of these sample were found positive. The findings are very similar to those made in the Gdańsk Deep.

Also here two types of degradation products were found in the area: sulphur mustard-related and those present in arsine oil including Clark I. Both samples contained both types of degradation products.

Mustard-related degradation product 1,4,5-oxadithiepane (**1.4**) and an arsenic-containing degradation product for phenyldichloroarsine (as **5T**).

Słupsk Furrow is in the area were the transport routes to both Gotland Deep and Gdańsk Deep could be passing through. Therefore, the timing of dumping and origin of the munitions in the area will be difficult to estimate.

3.3.6 Reference area

In order to get blank sediment samples and to capture fish in an area similar to the dump sites, a reference area was designated ca. 70 km north-west from Gdańsk Deep.

Unfortunately one of the three sediment samples taken in the area was positive. In this sample, degradation products for both sulphur mustard-related and those present in arsine oil including Clark I were found..

Mustard-related degradation products include thiodiglycol sulphoxide (**1.1O** analysed as silylated **1.1OS**) and two cyclic chemicals – 1,4-oxathiane (**1.3**) and 1,4,5-oxadithiepane (**1.4**). The arsenic-containing chemicals include triphenylarsine [both intact (**4**) and oxidised (**4O**)] and degradation product for phenyldichloroarsine (as **5T**).

Like Słupsk Furrow, the reference area could be along a transport routes to both Gotland Deep and Gdańsk Deep.

3.4 Repeated analysis of sediment samples

3.4.1 Sediment samples by VERIFIN

Ten of the sediment samples were selected for repeated analysis by VERIFIN. In this analysis, a new sediment fraction was taken from the original sediment. This means that the sample preparation procedure was fully repeated starting with the centrifuging of the wet sediment. The same samples were selected for reanalysis as had been selected for pore water sample analysis.

Sediment is not a homogenous matrix. Therefore, quite large differences could be expected from the repeated analysis samples. In general, the results of the repeated analysis were quite similar to those obtained originally. The results of the two analyses are compared in Table 8.

Most of the original findings were confirmed by the reanalysis of the samples.

Table 8. Comparison of the results (µg/kg dw) of original and reanalysed sediment fractions. Upper figure (in bold) is the original result and the lower figure is from the repeated analysis. *[from report by VERIFIN]*

Sample code	H				DM	DA		TPA		PDCA
	1	1.2	1.4	1.5	2O	3O	3T	4	4O	5T
WH349/B13/3	**(0.030)** (0.015)	**0.52** (0.20)	**2.8** 4.0	**28** 40	**210** 330	– –	**150** 2.0	**8.0** 2.3	– –	**4.0** 3.3
WH349/B13/7	– –	– –	– –	**(1.1)** (0.86)	– –	**68** 56	**19** 120	**5.5** 5.7	**–** 8.8	**41** 130
4SEP12	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
7Mar12	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
11GDapr12	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
2Apr13ROV	– –	– –	– –	– –	**22** 36	**1300** 930	– –	– –	**190** 340	– –
3Apr13BOX	– –	– –	– –	– –	**5.2** –	**1300** >200†	– –	– –	**180** >200†	– –
5Apr13ROV	– –	– –	– –	– –	**30** –	**210** 99	– –	– –	**67** 88	– –
6Apr13BOX	– –	– –	– –	– –	**30** 460	**35** 48	– –	– –	– 46	– –
14Apr13BOX	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –

* The sulphur mustard finding in sample WH349/B13/3 was well below quantitation limit, but it was identified unambiguously *(estimated amount in the sample was 0.030 µg/kg dw)*

† The result was outside the measurement range. Sample was not re-run as diluted.

3.4.2 Sediment core samples by MUT

As the two sediment core samples were the only core samples found to contain target chemicals and as thiodiglycol (**1.1**) was found in only these samples, MUT reanalysed these core slices in order to verify the correctness of the findings.

The results are presented in Table 9. Some differences are seen between the original results and the reanalysis results. This is, however, typical to the sediment samples as the distribution within one slice can vary greatly.

Table 9. Comparison of the thiodiglycol (**1.1**) results (µg/kg dw) of original and reanalysed fractions sediment cores *Q_7466* and *K_1857*. Each slice was divided in two portions (a and b). Upper figure (in bold) is the original result and the lower figure is from the repeated analysis. *[data by MUT]*

Slice	Q_7466		K_1857	
	a	b	a	b
0–2.5 cm	– n.a.	– n.a.	– n.a.	– n.a.
2.5–5 cm	– n.a.	– n.a.	– n.a.	– n.a.
5–7.5 cm	– n.a.	– n.a.	– n.a.	– n.a.
7.5–10 cm	– n.a.	– n.a.	– –	– –
10–13 cm	– n.a.	– n.a.	**19** 50	– –
12.5–15 cm	– n.a.	– n.a.	**20** 11	– –
15–18 cm	– n.a.	– n.a.	**14** –	– –
17.5–20 cm	– n.a.	– n.a.	– –	– –
20–23 cm	– n.a.	– n.a.	**18** –	– –
22.5–25 cm	– 7.1	**53** 20	**32** 8.6	– –
25–28 cm	– n.a.	– n.a.	– –	– –
27.5–30 cm	– n.a.	– n.a.	– n.a.	– n.a.

n.a. = not analysed

3.5 Total and inorganic arsenic concentrations in sediment

Three laboratories performed analysis of the arsenic concentration in sediment samples: IOPAN, MUT and LEPA. The analyses for LEPA were done in a subcontracting laboratory.

In total arsenic measurement was performed on 180 sediment samples. Some sediment samples were analysed only for arsenic (i.e. no analysis of CWA). IOPAN analysed all but two of the samples. MUT analysed 44 samples and LEPA 59 samples. In total, 88 samples were analysed by two laboratories and six samples by all three laboratories.

As already discussed in chapter 1.4 (Inter-calibration study), the total arsenic measurement was considered acceptable, but the there were doubts on performance of the extraction to remove organic arsenic chemicals from the samples. More information on this can be found in Attachment 6.

3.5.1 Validity of laboratories' analysis

As discussed under inter-calibration study (chapter 1.4) the values from the three laboratories were compared to each other to assess the validity of the data. Figure 26 shows the variation (from minimum to maximum) of the data origination from more than one laboratory.

Six samples analysed by all three laboratories are shown in Figure 27. The largest differences are shown in samples *26Apr13Box* and *WH349/B13/1*. In both cases, two other laboratories are quite close to each other. This difference could be due to inhomogeneity of the samples already seen in CWA analysis. In order to compare the overall matching of the results, results from MUT and LEPA were compared to those from IOPAN (see Figure 28). The both correlations are acceptable with correlations of 0.74 and 0.73. Based on these values the data from all laboratories seem valid.

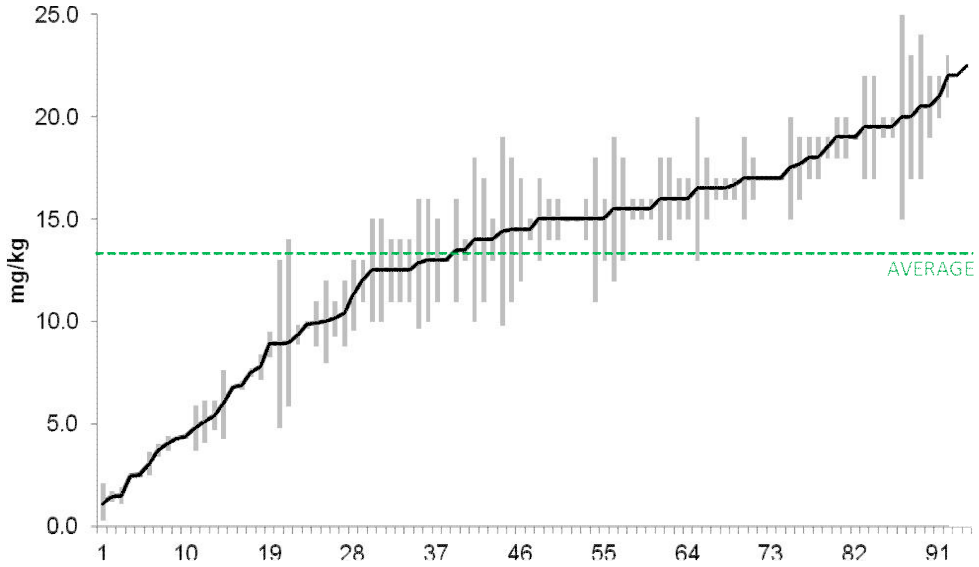

Figure 26. Variation of total arsenic measurements by different laboratories. Solid line is the mean of all measurements and grey bars show the distribution of results. The average arsenic in all samples was 13.3 mg/kg.

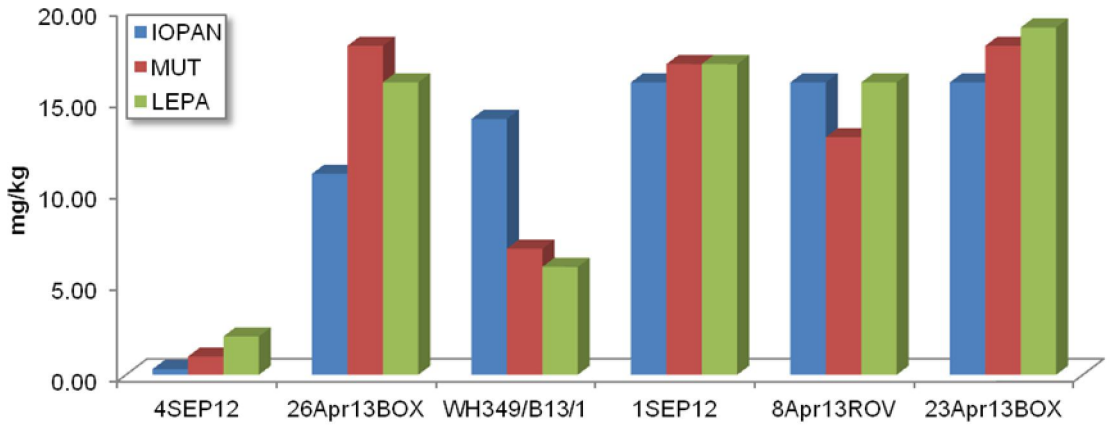

Figure 27. Comparison of total arsenic results for the samples received by all three laboratories.

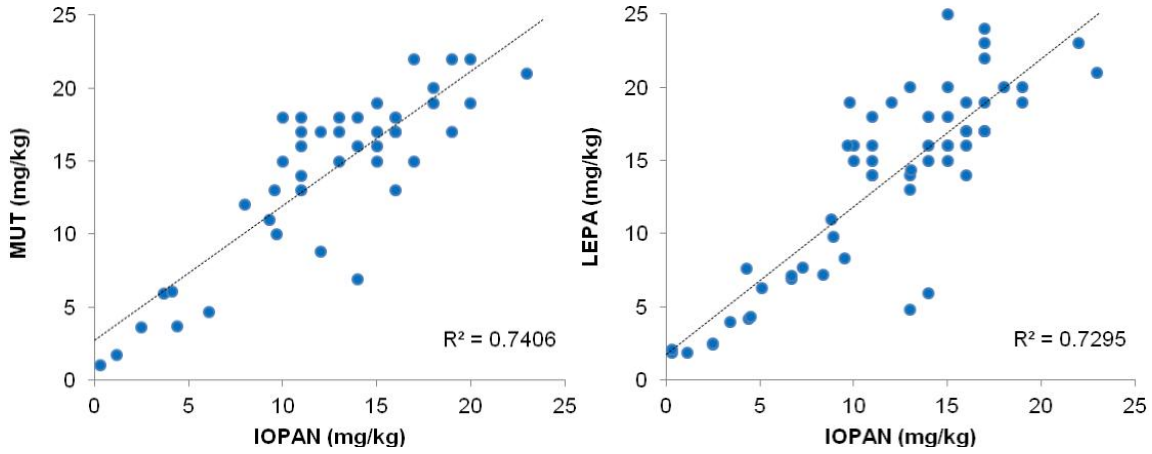

Figure 28. Correlation of total arsenic results from MUT (left) and LEPA (right) to results from IOPAN.

3.5.2 Results of the analyses

Based on literature, the geochemical arsenic background should not exceed 30 mg/kg. The highest results were below 25 mg/kg with only nine samples above 20 mg/kg. The average of all measurements was 13.6 mg/kg, while the median concentration was 14 mg/kg. Figure 29 shows the distribution of the arsenic concentrations in all analysed sediment samples. The average maximum difference between laboratories was 2.8 mg/kg with standard deviation of 2.4 mg/kg.

There does not seem to be any overall correlation between the total arsenic concentration and the CWA concentration. Figure 30 presents the distribution of total arsenic concentration in those samples where no CWAs were identified. The median of these samples was 13 mg/kg. On the other hand, the median of arsenic concentrations in samples where positive CWA identifications were made is 15 mg/kg as shown in Figure 31. *This small difference is not significant.*

There is a difference in total arsenic concentration in different areas as shown in Figure 32. The average total arsenic concentration in the sediment samples taken at the three dumpsites –

Bornholm, Gdańsk and Gotland Deep – are higher than the average concentration (Gotland only slightly).

The values from Gulf of Gdańsk vary greatly: the maximum concentration is 16 mg/kg while four samples have concentration of only 1–2 mg/kg. These low concentration have been measured at two or three different laboratories in same samples. The highest values are found in two samples close to the Hel Peninsula. Otherwise, the concentrations near the coast are low and increase towards the Gdańsk Deep as shown in Figure 33.

The three other areas – the reference area, Lithuanian part of Gotland Deep and the Słupsk Furrow – have clearly lower average arsenic concentration.

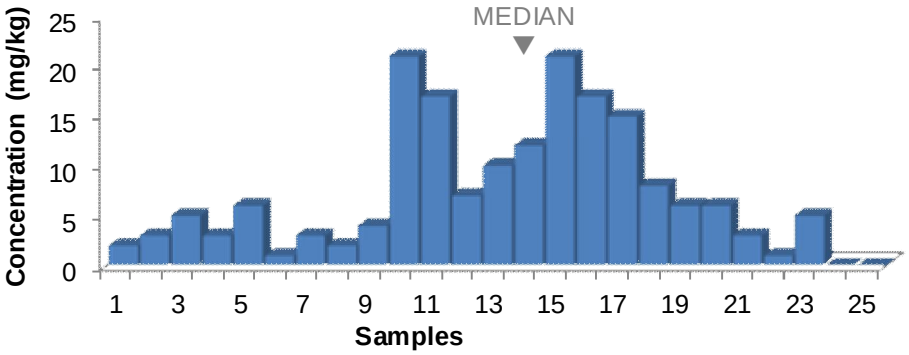

Figure 29. Distribution of total arsenic concentrations in **all sediment samples**. The median concentration of the arsenic findings in all samples was 14 mg/kg.

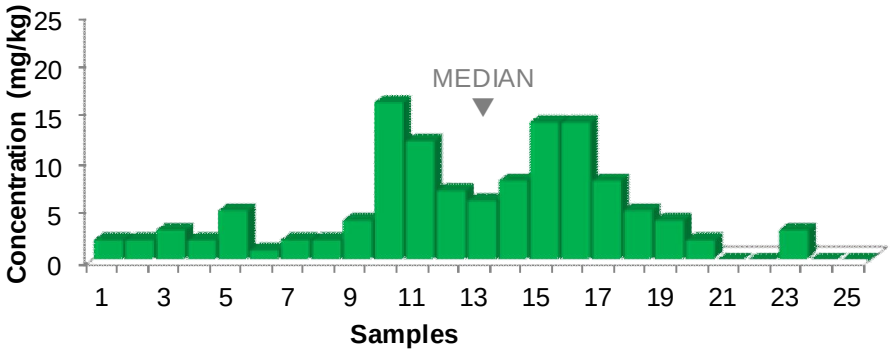

Figure 30. Distribution of total arsenic concentrations in samples with **negative findings** of CWA. The median concentration of the arsenic findings in these samples was 13 mg/kg.

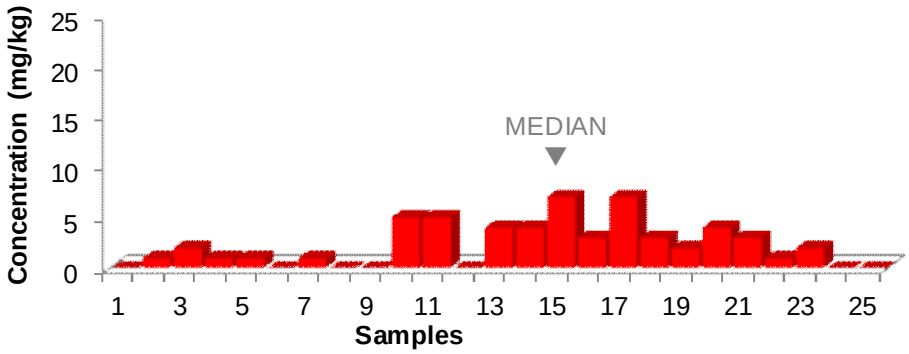

Figure 31. Distribution of total arsenic concentrations in samples with **positive findings** of CWA. The median concentration of the arsenic findings in these samples was 15 mg/kg.

The inorganic arsenic results (or the calculated organic arsenic) did not give any clear results. The organic arsenic had a correlation with the total arsenic values (r^2=0.75). The was also a correlation between total arsenic concentration and iron concentration (r^2=0.67) and total arsenic concentration and iron concentration (r^2=0.71). The correlation between total arsenic concentration and manganese concentration was clearly lower (r^2=0.36). Paka and Spiridonov have claimed that high arsenic concentration with increase of iron and manganese could point to existence of a localised source of arsenic, which in this case could mean presence of munitions or containers.

In summary total arsenic concentrations are slightly elevated in areas of chemical munitions dumping, however better knowledge of arsenic behaviour in marine environment is crucial for understanding the dependencies between its concentrations and occurrence of arsenic containing CWA. Investigation on specific arsenic forms is needed for recognition of processes of arsenic distribution.

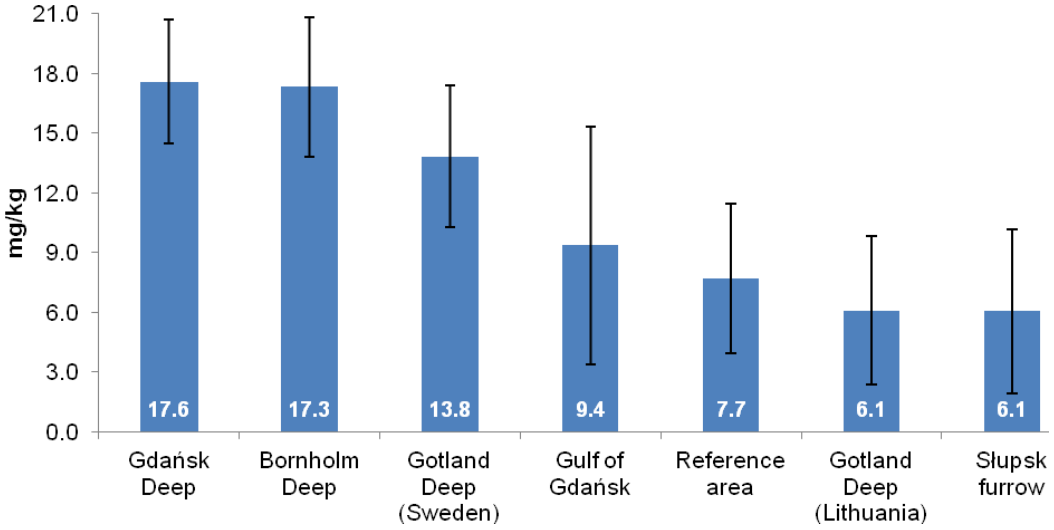

Figure 32. Distribution of total arsenic concentrations in different areas. Blue bars show the average concentration and error bars show the standard deviation.

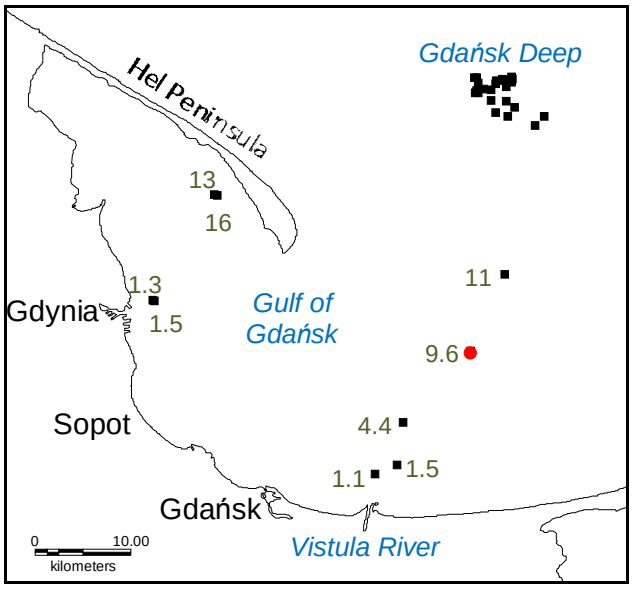

Figure 33. Arsenic concentrations in sediment samples in Gulf of Gdańsk. Sampling points are marked with ■. The sample with triphenylarsine finding (0.7 µg/kg) is marked with ●.

4. Conclusions

During the CHEMSEA project, 178 sediment samples were analysed for chemical warfare agents (CWA) and 180 sediment samples for arsenic. The analyses were performed in five different laboratories:

- Swedish Defence Research Agency (FOI)
  – 89 sediment samples and 11 pore water fractions for CWA
- Military University of Technology (MUT) in Poland
  – 73 sediment samples and 10 pore water fractions for CWA, 44 sediment samples for arsenic
- Finnish Institute for Verification of the Chemical Weapon Convention (VERIFIN)
  – 82 sediment samples and 10 pore water fractions for CWA
- Institute of Oceanology of the Polish Academy of Sciences (IOPAN)
  – 180 sediment samples for arsenic
- Lithuanian Environmental Protection Agency (LEPA)
  – 59 sediment samples for arsenic

In total, ca. 5900 individual analysis results for target were produced.

The analysis methods were tested before the analysis of actual sediment samples in an inter-calibration study. All the laboratories performing the CWA analyses also provided thorough validation data measured during the sample analysis.

Two known dumping sites (Bornholm Deep and Gotland Deep) and one suspected dumping site (Gdańsk Deep) were selected as the sampling sites. Additional sites within the Polish EEZ were also sampled: Gulf of Gdańsk and Słupsk Furrow as well as a reference site between Gdańsk Deep and Słupsk Furrow.

Positive CWA findings were made in all sampling areas. The high level of contamination was confirmed at Bornholm Deep, which is the largest dumpsite in Baltic. Bornholm dumpsite has been studied in a previous MERCW project. In CHEMSEA project, more emphasis was put on studying the previously less studied Gotland Deep with more than 80 sediment samples taken in the area. Dumping was confirmed in the area. The contamination was, as expected based on the amounts of dumped munitions, lower than in Bornholm dumpsite. Finally, a suspicion of CWA dumping at Gdańsk Deep was confirmed.

Target chemicals related to the following chemicals were identified:

- Sulphur mustard *(both intact and degradation products)*
- Adamsite *(degradation products)*
- Clark I *(degradation products)*
- phenyldichloroarsine *(degradation products)*
- triphenylarsine *(intact and oxidised)*
- α-chloroacetophenone *(intact)*

One sample was found to contain intact sulphur mustard. The concentration was well below the limit of identification (LOQ). The presence of intact agent in the sediment could be interpreted as evidence of recent release of mustard from a container such an artillery shell or an aerial bomb.

Although, several samples contained degradation products for sulphur mustard (**1**), there were clearly fewer findings at lower concentrations than for arsenic-containing chemicals. Still, mustard is the most dumped agent in Baltic Sea. Several hypotheses for the low amount of mustard-findings can be presented:

- Mustard is in a form not detected in the current analysis. This would mean that there could be still identified abundant degradation products in sediment. The degradation of mustard in sediment should be studied in laboratory studies.
- Mustard is not released from munitions/containers and subsequently spreading to the sediment. This could be due to formation poorly soluble or totally insoluble lumps captured by fishermen.

- Mustard is either not bound or is removed from the sediment surrounding the dumped munitions and containers. This could occur e.g. by hydrolysis and subsequent dissolution in sea water and dilution beyond detection. Also, it has been speculated that bacteria could consume thiodiglycol in the sediment.

A wide range of target chemicals had been included for both mustard-related and arsenic-containing chemicals. Based on the results, it seems that only target chemical, which is not necessary as it has not been found in any of the samples is the cyclic degradation product, 1,7-Dioxa-4,10-dithiacyclododecane (**1.6**), for sulphur mustard. Also, derivatisation of Adamsite-related degradation product using propane-1-thiol did not work well. The oxidised derivative produced much better results, although it must be analysed using an LC–MS instrument.

Most of the positive findings (42 samples) were caused by degradation products of arsenic-containing chemicals originating from Adamsite, Clark I and arsine oil. The maximum concentrations of these chemicals were over 1000 µg/kg dw in Bornholm Deep and over 200 µg/kg dw in Gotland and Gdańsk Deeps.

Total arsenic concentrations of sediment samples did not have any correlation to CWA findings. However, different sampling areas had quite different average arsenic concentrations. Clearly elevated arsenic concentrations were found in Gdańsk and Bornholm Deep areas. The Swedish part of the Gotland Deep had concentration slightly above the average, while the Lithuanian part had clearly lower than average arsenic concentration. Gulf of Gdańsk, the reference site and Słupsk Furrow had the lowest arsenic concentrations.

More information is needed better understand the behaviour of arsenic in marine environment. Currently, it is quite difficult to dependency of total and inorganic arsenic to the presence of arsenic-containing CWA. Investigation of specific arsenic species in the sediment is required to understand these depencies.

Based on the results of the sediment samples, 23 pore water portions of sediment samples were selected for pore water analysis. Out of these samples, 18 were positive. The most remarkable finding in the pore water analysis is that degradation products of Clark seem to be transferring to pore water in relatively high amounts. This could mean that these chemicals could be more readily available for transfer into surrounding sea water and therefore likely to spread to the neighbouring areas and/or to transfer to organisms close to the contaminated area.

The potential risk posed by the degradation products for Clark I should be studied further. These chemicals are widely spread in the sediment in different dumping sites and they seem to be present in the pore water. They have also been shown to have adverse effects at least on mammals.

All three dumpsite areas – Bornholm, Gotland and Gdańsk Deeps – were found to contain degradation products originating from dumped munitions. The majority of the contamination in all three areas was due to arsenic-containing chemical warfare agents. In Bornholm and Gdańsk Deeps, the level of arsenic in the sediment is clearly elevated and in Gotland Deep higher than the average. Gotland Deep was studied most in the CHEMSEA project and Bornholm had been already studied in MERCW project during 2006–2008. Next focus area should be the Gdańsk Deep, which has now been confirmed as a dumpsite.