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Identification of complexes formed between sulphur mustards and arsenic-containing chemical warfare agents

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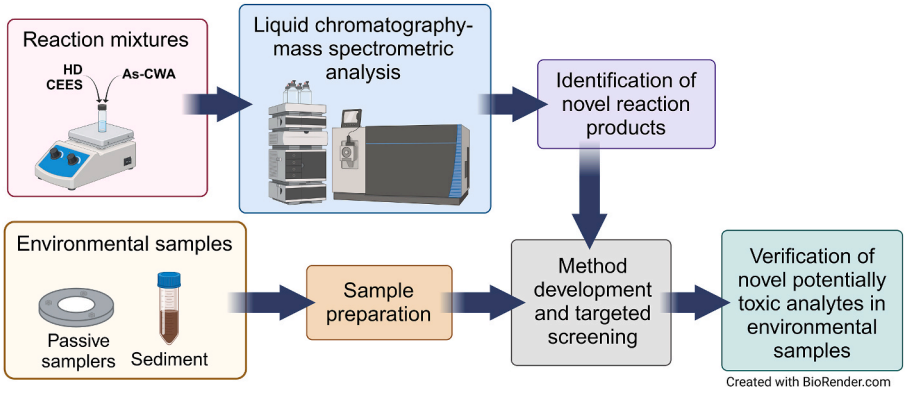
HIGHLIGHTS

- Reactions of S-mustards with As-CWAs were studied using UHPLC-HRMS analysis.
- 22 novel complexes were identified in reactions between HD or CEES and As-CWAs.
- 8 As-CWA-HD-complexes were found in samples collected from the Baltic Sea region.
- For the first time, alkylation of As-CWAs by S-mustards is reported.
- The novel complexes are potentially toxic to humans and marine biota.

GRAPHICAL ABSTRACT



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ABSTRACT

Large quantities of toxic chemical warfare material, such as mixtures of sulphur mustard (HD) and arsenic-containing chemical warfare agents (As-CWAs) have been submerged in the Baltic Sea region after World War II. Little is known about the possible reactivity between HD and As-CWAs. In this study, we used simple reaction mixtures and ultra-high performance liquid chromatography combined with mass spectrometric techniques to study the reactivity of HD and the half-mustard 2-chloroethyl ethyl sulphide (CEES) with As-CWAs. Altogether 22 novel As-CWA-HD- and As-CWA-CEES-complexes were identified. Eight of the As-CWA-HD-complexes were also detected in environmental samples collected from known CWA dumping sites in the Baltic Sea region. Because the As-CWA-HD- and As-CWA-CEES-complexes have structural moieties of both S-mustards and As-CWAs, they might have toxic properties of both CWA-types. Therefore, their occurrence in the environment is concerning and their potential negative effect on the wellbeing of marine biota and humans should be investigated in the future. This is the first time alkylation of As–CWAs is reported, providing new knowledge on the reactivity of S-

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mustards. The results increase the understanding of the environmental fate of HD and is valuable for the assessment of the environmental threat of sea-dumped CWAs.

1. Introduction

In the aftermaths of World War II, large quantities of chemical weapons (CWs) and chemical warfare agents (CWAs) were disposed of by dumping in seas (Radke et al., 2014), (GLASBY, 1997). In Europe, the dumping was mainly done in the Baltic Sea and in Skagerrak (Baltic Sea region). According to estimations, 40 000 and 160 000 tonnes of chemical warfare was submerged at these sites, respectively (HELCOM et al., 2013), (Tørnes et al., 2020). The majority of the dumped chemical warfare material was filled with bis(2-chloroethyl) sulphide, also known as sulphur mustard (HD) (Fig. 1) (HELCOM et al., 2013), (Tørnes et al., 2020), (Tørnes et al., 2006). In addition to HD, arsenic-containing CWAs (As-CWAs) were dumped in Skagerrak and in the Baltic Sea (HELCOM et al., 2013), (Tørnes et al., 2020). The dumped As-CWAs include Adamsite (DM), phenyldichloroarsine (PDCA), Clark I (DA), Clark II (DC) triphenylarsine (TPA) and Lewisite, which is a mixture of 2-chlorovinylarsine chloride (Lewisite 1, L1), bis(2-chlorovinyl)arsine chloride (Lewisite 2, L2), and tris(2-chlorovinyl)arsine (Lewisite 3, L3) (Fig. 1). In the marine environment HD and As-CWAs hydrolyse and oxidise to the corresponding hydroxides, oxides and acids, which are often referred to as primary degradation products. The CWAs and their primary degradation products can also be transformed through (bio)chemical processes to new chemicals (Söderström et al., 2018), (Hemström et al., 2020), (Wang et al., 2012), (Niemikoski et al., 2020a), (Rantanen et al., 2024), (Niemikoski et al., 2020b), (Niemikoski et al., 2021), (Chmielińska et al., 2019), (Zalewska et al., 2023). In this paper the term transformation product is used to refer to all chemicals formed from the CWAs through metabolism and chemical reactions.

When HD comes in contact with water in the environment, it rapidly hydrolyses to thiodiglycol (TDG), which can further oxidise to thiodiglycol sulphoxide (TDGO) and thiodiglycol sulphone (TDGS) (Söderström et al., 2018), (Valdez et al., 2018). Due to its high reactivity, HD undergoes intramolecular cyclisation reactions producing cyclic transformation products, like 1,4-dithiane, 1,4-oxathiane, and 1,2,5-trithiepane (Söderström et al., 2018), (Hemström et al., 2020), (Wang et al., 2012), (Ashmore and Nathanail, 2008), (Chmielińska et al., 2019), (Czub et al., 2020). Intermolecular cyclisation or polymerisation reactions can also produce solid polymeric mustard (mustard heel) and semi-solid aggregates of mustard (lumps). Both mustard heel and lumps can have intact HD or other sulphur mustards (S-mustards) trapped inside them, and can cause accidental exposure of, for example, fishermen to S-mustards (HELCOM et al., 2013), (Söderström et al., 2018), (Hemström et al., 2020), (Chmielińska et al., 2019), (Czub et al., 2020). As-CWAs are hydrolysed and oxidised in the environment to the corresponding arsenic acids: 10-hydroxy-5H-phenarsazine 10-oxide (DM [ox]); phenylarsinic acid (PAA); diphenylarsinic acid (DPAA); triphenylarsine oxide (TPAO); 2-chlorovinylarsinic acid (CVAOA); bis(2-chlorovinyl)arsinic acid (b-CVAOA); and tris(2-chlorovinyl)arsine oxide (t-CVAO) (Fig. 1). The hydrolysis and oxidation reactions of As-CWAs occur through a di- or polymeric intermediate, which is why bis(diphenylarsine) oxide can sometimes be detected in the environment (Söderström et al., 2018), (Niemikoski et al., 2017a), (Ishii et al., 2004). Recent studies have also shown that phenylarsenic CWAs and their primary degradation products are transformed to hydroxyl-, methyl- and sulphur-containing phenylarsenicals (Niemikoski et al., 2020a), (Rantanen et al., 2024). These transformation products are produced through direct and indirect microbial metabolism in complex equilibrium reactions determined by the chemical conditions in the environment (Rantanen et al., 2024).

Because CWAs are highly toxic chemicals, the sea-dumped CW-material are a potential threat to marine ecosystems and humans utilising them. During the previous two decades, the occurrence of HD, As-CWAs and their transformation products in the marine environment have been investigated in several international projects, including “Modelling of Ecological Risks Related to Sea-Dumped Chemical Weapons” (MERCW), “Chemical Munition Search and Assessment” (CHEMSEA), and “Decision Aid for Marine Munitions” (DAIMON) I and II (Fauser et al., 2023), (Czub et al., 2024), but many un-answered questions still remain about the short- and long-term toxicity of CWAs and their fate in the environment. To get a better understanding of the environmental threat of sea-dumped chemical warfare material, one of the many goals of the projects has been to analyse sediment and marine biota for the occurrence of CWAs and their transformation products (Fauser et al., 2023). CWAs and their transformation products are usually analysed from

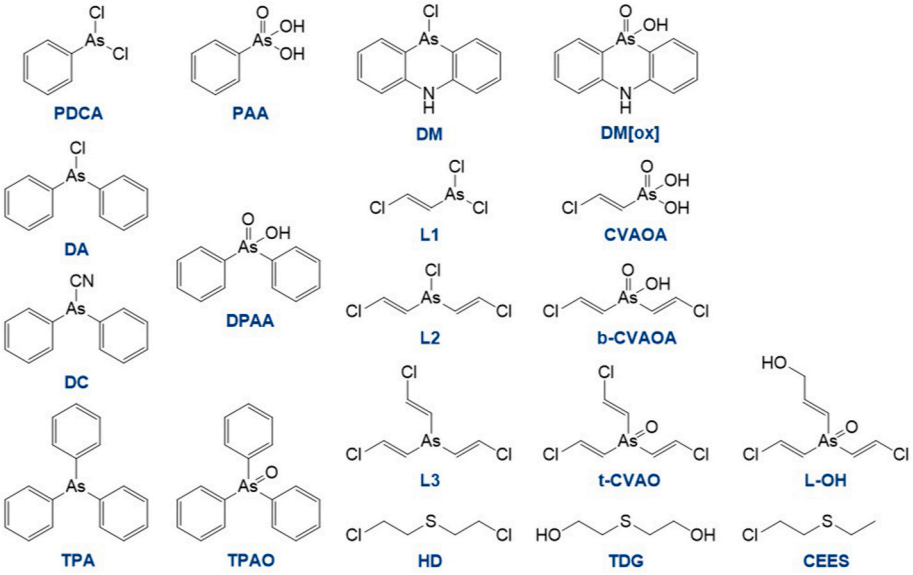


Fig. 1. CWAs and their primary degradation products considered in this work.

environmental samples, using gas and liquid chromatography (GC and LC, respectively) coupled with mass spectrometric (MS) techniques (Vanninen et al., 2023). HD and the cyclic degradation products can be analysed by GC-MS directly, but the polar degradation products require chemical derivatisation with, for example, N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) or N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) (Valdez et al., 2018), (Vanninen et al., 2023), (Söderström and Östin, 2017). LC-MS can be used to analyse degradation products of HD without derivatisation (Valdez et al., 2018), (Vanninen et al., 2023), (Söderström and Östin, 2017). The transformation products of the As-CWAs can be directly analysed by LC-MS techniques. The intact As-CWAs are analysed with GC-based techniques after derivatisation to more volatile forms. Arsenic (As) has a strong affinity to sulphur, which is why thiols are often used to derivatise As-CWAs and their degradation products for GC analysis (Tørnes et al., 2006), (Valdez et al., 2018), (Söderström and Östin, 2017), (Sokołowski and Konopski, 2007), (Sokołowski et al., 2008), (Haas, 1998), (Stan'kov et al., 2011). Any thiol can be used to derivatise As-CWAs, allowing optimisation of the chromatographic separation and to minimise matrix interferences. However, the derivatisation yield can vary between different thiols and As-CWAs (Tørnes et al., 2006). Alcohols have been used as alternative derivatisation agents in the analysis of Lewisites by GC (Valdez et al., 2018), (Sokołowski and Konopski, 2007), (Sokołowski et al., 2008), (Stan'kov et al., 2011), (Epure et al., 2010), (Haas and Krippendorf, 1998). Monoalcohols with one to eight carbons and some dialcohols, including TDG, have been tested. All alcohols quickly produced the corresponding As-ethers with the Lewisites after incubation at room temperature (Haas, 1998), (Stan'kov et al., 2011), (Haas and Krippendorf, 1998) and at elevated temperatures have also been applied in some studies (Sokołowski and Konopski, 2007), (Sokołowski et al., 2008). Although the reactions between As and alcohols is fast, the ether derivatives formed in the reaction are unstable. The derivatives of the C1–C4 alcohols are especially unstable, degrading within a few hours to polar As-compounds that cannot be detected by GC-based techniques (Sokołowski and Konopski, 2007), (Sokołowski et al., 2008), (Epure et al., 2010), (Haas and Krippendorf, 1998). TDG produced slightly more stable derivatives, leading the authors to suggest that Lewisites could be used as alternative derivatisation agents for GC-analysis of TDG (Sokołowski and Konopski, 2007), (Sokołowski et al., 2008).

In the aforementioned projects, the primary degradation products of the phenylarsenic-CWAs (DM[ox], PAA, DPAA, and TPAO) and their methyl- and/or sulphur-containing analogues, have been identified in marine sediments collected from the Baltic Sea region (HELCOM et al., 2013), (Tørnes et al., 2006), (Niemikoski et al., 2020a), (Rantanen et al., 2024), (Barbosa et al., 2023). The only intact As-CWA found in sediment is TPA, which is detected presumably because of its hydrophobicity and slow degradation rate (Söderström et al., 2018). DPAA and TPAO have also been detected in marine biota caught in Bornholm and Skagerrak (Niemikoski et al., 2017b), (Niemikoski et al., 2020c), (Niemikoski, 2022). Laboratory experiments and modelling have shown that As-CWAs and/or their degradation products accumulate in mussels and fish, and are toxic to water fleas and fish (Niemikoski et al., 2021), (Czub et al., 2024), (Höher et al., 2019), (Czub et al., 2021), (Wilczynski et al., 2023), (Wilczynski et al., 2024). Lewisites and their degradation products have rarely been found in samples collected from dumping sites (Bełdowski et al., 2016). This is expected, since only small amounts of chemical warfare material containing Lewisites have been dumped in the Baltic Sea region (Radke et al., 2014), (GLASBY, 1997), (HELCOM et al., 2013), (Tørnes et al., 2020). The primary degradation products of HD (TDG, TDGO and TDGS) are seldom found in sediments collected from the Baltic Sea region. Instead, cyclic transformation products, like 1,4-dithiane, 1,4-oxathiane, and 1,2,5-trithiepane, are typically found. Intact HD has also been detected in sediment extracts, which is interesting considering how quickly HD hydrolyses in water (Söderström et al., 2018), (Bełdowski et al., 2016). HD has poor solubility in water (Ashmore and Nathanail, 2008), so the agent leaking from the containers might form droplets suspended in the sediment and the near-bottom water. Similarly, as in mustard lumps, the surface of the droplets is passivated by the intramolecular polymerisation reactions, trapping intact HD inside them (Söderström et al., 2018). Both laboratory experiments and field work have been conducted to investigate whether HD or its transformation products bioaccumulate in shrimp, eel and fish. No HD-related chemicals were found in these studies (Della Torre et al., 2013), (Koide et al., 2016), (Della Torre et al., 2010). However, experiments have shown that both HD and its degradation products produce a toxic response in eel, fish, water fleas, algae and human cells (Hemström et al., 2020), (Chmielińska et al., 2019), (Czub et al., 2020), (Della Torre et al., 2013), (Della Torre et al., 2010), (Huong et al., 2023).

While As-CWAs and their degradation products have been detected in marine sediments on various occasions, the amounts and detection frequency of HD and its degradation products are too low compared to the known quantity of HD submerged in the Baltic Sea region (Söderström et al., 2018), (Hemström et al., 2020), (Bełdowski et al., 2016). This is a clear indication that the knowledge about the environmental fate of HD is limited. The hydrolysis and intra- and intermolecular polymerisation reactions mentioned above, are important in the transformation of HD in the environment (Chmielińska et al., 2019), (Ashmore and Nathanail, 2008). The rate of the hydrolysis is determined by how efficiently HD is dissolved in water (Ashmore and Nathanail, 2008), (Czub et al., 2020). Due to the hydrophobic nature of HD, it forms oily droplets or semi-solid lumps in water (Ashmore and Nathanail, 2008), (Czub et al., 2020), (Huong et al., 2023). Polymerisation reactions passivate the surface of the droplets and the lumps, which slows down the hydrolysis and hinders the HD from spreading in to the surroundings (Ashmore and Nathanail, 2008), (Czub et al., 2020), (Bełdowski et al., 2016), (Huong et al., 2023). In addition, HD may interact with silica particles and organic matter, binding it to the solid phase of the sediment (Chmielińska et al., 2019), (Ashmore and Nathanail, 2008). The polymerisation and binding to solid particles results in a localised contamination which is difficult to locate with grab sampling (the sampling technique used in most studies so far). This may explain why HD-related chemicals are not found as often as one would assume based on the reported quantities of dumped HD (Barbosa et al., 2023). Metabolic transformation of the half-mustard 2-chloroethyl ethyl sulphide (CEES) has been reported, which suggests that HD may also be transformed by microbes in the sediment (Ashmore and Nathanail, 2008). However, HD is a highly toxic chemical, so high concentrations of HD leaking from the CWs might kill the microbial communities before they have time to transform the agent. On the contrary, several microbes that are able to utilise TDG in their metabolism have been reported (Ashmore and Nathanail, 2008), (Medvedeva et al., 2012), (Medvedeva et al., 2009). Thus, microbial transformation could partially explain the low occurrence of HD-related chemicals in known dumping sites.

Another possible explanation for the low quantity and detection frequency of HD-related chemicals, in samples collected from marine dumping sites, is the reactivity of HD with other chemicals in the munition forming yet unidentified transformation products. Historically, HD used in weapons was typically not pure and often mixed with other substances, such as thickening agents and other CWAs, to enhance its usability and to increase its immobilising effect (Radke et al., 2014), (Söderström et al., 2018), (Ashmore and Nathanail, 2008), (Chmielińska et al., 2019), (Epure et al., 2010), (Muzaffar et al., 2023), (Isono et al., 2018). An example of this is winter-grade HD, which was designed for use in cold climates. It contained As-CWAs, which lowered the freezing point of HD. Additionally, the As-CWAs penetrated gas masks, resulting in violent sneezing and coughing. This in turn forced soldiers to remove their gas masks, subsequently causing exposure to HD through the eyes and respiratory system (Radke et al., 2014), (Söderström et al., 2018), (Chmielińska et al., 2019), (Epure et al., 2010), (Muzaffar et al., 2023), (Isono et al., 2018). During World War II, Germany mixed HD with

phenylarsenic CWAs (DM, PDCA, DA, DC, TPA), while the USA, the Soviet Union and Japan mixed it with Lewisite (L1, L2, L3) (Söderström et al., 2018), (Epure et al., 2010), (Isono et al., 2018). Although the combined use of HD with As-CWAs is known, little work has been done to investigate how the additives affect the chemical properties, degradation in the environment, and toxicity of HD (Ashmore and Nathanail, 2008), (Czub et al., 2021).

In the present work, the reactions between HD and As-CWAs were investigated further. To our knowledge, only one study on the possible reactivity between these agents has been published (Opstad, 2015). In the study, HD was mixed with phenylarsenic oxide (PAO, a degradation product of PDCA), DA and trichloroarsine (TCA) in seawater, incubated for 1 day to 6 months and analysed with GC-MS after extraction. Using spectral interpretation, retention indexes and library matching three PAO-related compounds, where the mustard had bound to the As-atom through the oxygen- or sulphur-atom (PAO-ethers and PAO-thioethers, respectively) were identified. The structural similarities between the PAO-(thio)ethers and the previously reported thiol- and TDG-derivatives of L1 (Sokołowski and Konopski, 2007) suggests that the arsenical may have reacted with degradation products of HD rather than with the intact agent (Opstad, 2015). Considering the high reactivity of HD, it is interesting that no HD-complexes of PAO, DA and TCA were found in the study. The reactivity of HD is based on the formation of a sulphonium-ion, which rapidly reacts with almost any available nucleophile (Valdez et al., 2018), (Bae and Winemiller, 2013) (Figure S.1 in Supplementary information (SI)). In aqueous environments, the most prominent compounds containing nucleophiles are the water molecules (reaction with water produces TDG), and the additives mixed in the munition, such as As-CWAs. Based on this and As' affinity to sulphur, we hypothesise that HD can react with As-CWAs to form As-CWA-HD-complexes. Because the three PAO-(thio)ethers identified previously were detected in Skagerrak sediments (Opstad, 2015), we also believe that the As-CWA-HD-complexes can be found in the environment. Previous studies on CWAs dumped in the Baltic Sea region have focused on analysing the primary degradation products of HD and As-CWAs (Tørnes et al., 2006), (Söderström et al., 2018), (Fauser et al., 2023), (Söderström and Östin, 2017), (Bełdowski et al., 2016), suggesting that other transformation and reaction products may have gone undetected. Therefore, the formation of As-CWA-HD-complexes in the sea-dumped CWs, could provide an additional explanation to the unexpectedly low quantities of HD-related chemicals detected in the environmental samples collected from dumping sites in the Baltic Sea region.

In this study, the possible reactivity between HD and As-CWAs using simple reaction mixtures and ultra-high performance liquid chromatography (UHPLC) high-resolution (tandem) mass spectrometric (HRMS and MS/HRMS) analysis was investigated. To see whether other S-mustards react analogously, a structurally similar and less reactive half-mustard, CEES, was also tested (Fig. 1). A targeted UHPLC-MS/MS method was used to study the occurrence of the identified reaction products in sediment and passive samplers collected from CWA dumping sites and uncontaminated reference areas. A flowchart of the experimental design is presented in Figure S.2 in SI. The aim of this study is to produce new knowledge on the transformation of HD that may in part explain why less HD-related chemicals are found in the Baltic Sea region than expected. We also aimed to identify novel transformation products and to deepen the understanding of the reactivity and environmental fate of HD and As-CWAs. The knowledge produced in this work can be utilised in the environmental risk assessments of sea-dumped CWs and CWAs.

2. Experimental

2.1. Chemicals and reagents

High-performance LC- (HPLC) (≥99.5%) and LC-MS-grade (≥99.9%) acetonitrile (ACN) were purchased from Honeywell (Charlotte, NC, USA). HPLC-grade methanol (MeOH) and acetone were bought from Fisher Scientific (Hampton, NH, USA). Ultra-pure water was produced with a Millipore Direct-Q®3 UV system (Merck; Darmstadt, Germany). LC-MS-grade formic acid (FA, 99.9%) was bought from Sigma-Aldrich (Darmstadt, Germany). Technical grade HD (95%) and L1 (90%) were obtained from the Finnish Defence Research Agency (Lakiala, Finland) and L2 (97%) from Spiez Laboratory (Spiez, Switzerland). The L1 and L2 stocks contained L1, L2, L3 and other chlorovinylarsine-compounds as impurities. DA (99.9%; Envilytix Gmbh; Weisbaden, Germany), TPA (98%; Acros organics; Charlotte, NC, USA), PAA (97%; Acros organics), DPAA (99%; Envilytix Gmbh), TPAO (99.7%; Sigma-Aldrich), and CEES (95%; Acros organics) were bought from commercial suppliers. PDCA (60%), DC (80%), DM (87%), and TDG (90%) were synthesised in-house at the Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN). 2-Chlorovinylarsenic acid (CVAOA), bis-2-chlorovinyl)arsenic acid (b-CVAOA) and tris-(2-chlorovinyl)arsenic oxide (t-CVAO) were prepared by oxidation of L1 and L2 with 33% hydrogen peroxide (H_2O_2; VWR International; Radnor, PA, USA). Analytical grade anhydrous sodium sulphate (Na_2SO_4; 99%) was bought from Merck (Darmstadt, Germany).

2.2. Instrumentation

High-resolution screening and identification of reaction products were performed using a Thermo Scientific Fusion™ Orbitrap mass spectrometer (HRMS) coupled with a Thermo Scientific Dionex Ultimate 3000 ultra-high-performance liquid chromatographic (UHPLC) system (Waltham, MA, USA). The instrument was calibrated to ≤5 ppm mass accuracy by external calibration. The HRMS was equipped with a heated electrospray ionisation (HESI) ion source. The positive polarity spray voltage was set at 3500 V, vaporiser temperature at 300 °C, and ion transfer tube temperature at 250 °C. Sheet, auxiliary, and sweep gas flows were 40, 15, and 0 (arbitrary units), respectively. Mass scan was done over the mass-to-charge (m/z) range of 70–700 with 60 000 resolution (at m/z 200) and RF lens value 60%. UHPLC-tandem high-resolution mass spectrometry (MS/HRMS) with stepped high-energy collisional dissociation (HCD) was used to produce fragmentation patterns of interesting ions. Mass resolution in the UHPLC-MS/HRMS methods was 120 000. Collision energies in the stepped HCD fragmentation were typically set at 15%, 30%, and 45% (arbitrary units). Energies of 5%, 10%, and 15% were used for analytes present in very low concentrations. Xcalibur™ 4.5 software (Thermo Fisher Scientific; Waltham, MA, USA) was used to control the UHPLC-HRMS system and for data processing.

The chromatographic separation was achieved using a Waters™ Aquity UPLC®BEH C18 (2.1 × 100 mm, 1.7 μm) analytical column at 45 °C. The mobile phase consisted of 0.1% FA in ultra-pure water (eluent A) and 0.1% FA in ACN (eluent B). The following gradient was used in the analysis of synthesis products and sediment samples: linear increase from 5% to 100% B in 3.5 min, held at 100% B for 1 min, linear decrease to 5% B in 0.2 min, and held at 5% B for 0.8 min (5.5 min in total). Passive sampler extracts were analysed with a 7 min gradient. For the first 0.5 min the eluent contained 5% B, then the amount of eluent B was linearly increased to 100% in 4.5 min, where it was held for 1.5 min. The amount of eluent B was linearly decreased back to 5% in 0.5 min and held for 0.5 min. Flow was set at 0.5 ml/min in both methods.

A multiple reaction monitoring (MRM) method for targeted screening was set up based on the identified chemicals utilising the synthesis products. For the MRM analysis a Waters™ Xevo® TQ-XS triple quadrupole mass spectrometer equipped with a Waters™ Aquity I-class UPLC® separation system (Milford, MA, USA) was used. The mass spectrometer was equipped with an electrospray ionisation (ESI) source. The source temperature was 150 °C, capillary voltage was 3000 V, cone voltage was 20 V, desolvation temperature 500 °C, and desolvation gas (N_2) flow 1000 l/h. The analytical column, column temperature and

mobile phases were the same as in the high-resolution analysis. The separation was done using the 5.5 min gradient described before. The UHPLC-MS/MS system was operated with MassLynx 4.2 software (Waters™).

The performance of the UHPLC-MS/MS and UHPLC-HRMS instruments was tested daily using an in-house quality control sample designed to fulfil the criteria for accredited qualitative analysis of CWAs with requirements set by ISO/IEC 17025:2017. A control sample containing As-CWA-HD-complexes was used to monitor the performance of the targeted MRM-method. Solvent blanks were also analysed daily to monitor potential instrument contamination.

2.3. Reactivity studies and synthesis of reference chemicals

For the experiments, approximately 1 mg/ml stocks of the As-CWAs (TPA, DA, DC, PDCA, DM, L1 and L2), HD and the half-mustard CEES were prepared in acetone. To study the reactions, 20 μl of the HD stock was combined with 20 μl of one of the As-CWA stocks and diluted to 200 μl with ultra-pure water. The reaction mixtures were vortexed and left at room temperature (22 °C) to react overnight (7 reaction mixtures). The final concentration of the reaction mixtures was 0.1 mg/ml. For comparison, the reactivity of the half-mustard CEES was also tested by preparing similar reaction mixtures of CEES with the As-CWAs (7 reaction mixtures). Additional experiments were performed with selected degradation products to investigate differences between the reactions of intact CWAs and their degradation products. The degradation products selected were TDG, TPAO, DPAA and b-CVAOA. 1 mg/ml stocks of the degradation products were prepared in acetone. Reaction mixtures between the intact CWAs and/or degradation products were prepared as described before. The reaction mixtures prepared were: HD + TPA, HD + TPAO, TDG + TPA, TDG + TPAO, HD + DA, HD + DPAA, TDG + DA, TDG + DPAA, HD + L2, HD + b-CVAOA, TDG + L2, and TDG + b-CVAOA (12 reaction mixtures in total). Once the reaction mixtures had been incubated overnight, all 26 reaction mixtures were analysed with UHPLC-HRMS and UHPLC-MS/HRMS. The reaction mixtures prepared are summarised in Table S.1 in SI.

Attempts were made to synthesise compound A (Table 1) in larger scale for purification and analysis with nuclear magnetic resonance (NMR) spectroscopy. An amount of 0.05 mol of TPA was dissolved in 0.75 ml of ACN and two equivalents (0.1 mol) of HD was added. Under constant stirring, an amount of 2.25 ml of ultra-pure water was gradually added to the reaction over 24 h with an automatic glass syringe. Soon after the addition of water was started, white precipitate started to form in the reaction vessel. Once all the water had been added, the reaction mixture was stirred overnight at room temperature (22 °C) and ambient pressure. Then the stirring was stopped and the phases were allowed to settle, after which the liquid phase was collected in a clean vial. A sample of the solid phase was dissolved in ACN and diluted with ultra-pure water for UHPLC-HRMS analysis. Based on the analysis, the precipitate consisted mainly of TPAO. A sample of the liquid phase was diluted with ultra-pure water and analysed with UHPLC-HRMS. Compounds A–I, TPAO, and TDG were detected in the liquid phase sample. The intensities suggested that compound A was the most prominent analyte in the liquid phase, but isolating it from the crude product for NMR analysis was not successful.

2.4. Environmental samples

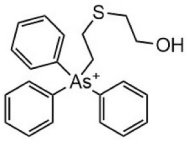
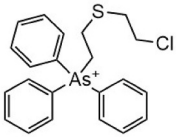
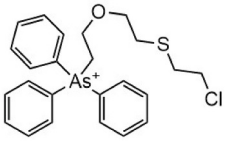
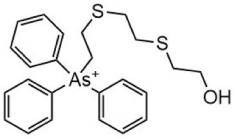
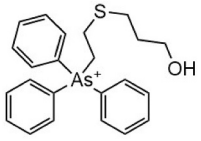
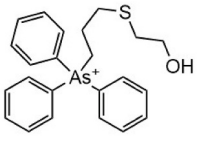
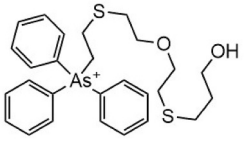
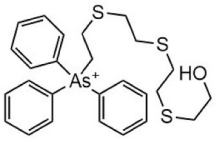
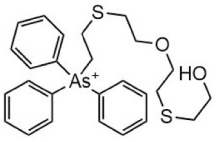
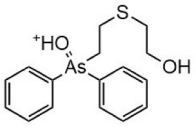
Two types of environmental samples collected from known CWA-dumping sites were analysed for the novel complexes identified from the reaction mixtures. The environmental samples consisted of Baltic Sea sediment, and passive samplers deployed in Skagerrak near the wreck of a ship that had been loaded with CW-material. No sediment samples from Skagerrak dumping site were available for this study. Methods described in the recommended operating procedure "Analysis of sediment samples for sea-dumped chemical weapons" (Söderström and Östin, 2017) were applied in the extraction of the sediments (section 2.4.1). Slightly modified sample preparation methods were used in the extraction of the passive samplers (section 2.4.2).

2.4.1. Sediment samples (the WARTOX-project)

The Baltic Sea sediments utilised in this study were collected for the project "Chemical warfare agents in the Baltic Sea: biodegradation, metabolism and toxicity (WARTOX)". The sediments had been collected from Bornholm dumping site (55.19568 N, 15.38022 E) and an uncontaminated reference area (JML; 59.58185 N, 23.62683 E). During the WARTOX-project 276 sediment samples (25 ± 2 g) were prepared (138 for Bornholm and 138 for JML) to study the transformation of HD (unpublished data) and selected As-CWAs (PDCA, DA and DM) (Rantanen et al., 2024) in marine sediments under oxic and anoxic conditions. Half of the samples of both sampling sites were weighed in a glove box constantly flushed with N_2 gas (anoxic conditions), and the other half was weighed in a regular glove box (oxic conditions). For both sampling sites, six unspiked control samples were prepared: three for incubation in oxic conditions and three for in anoxic incubation. The remaining sediment samples were spiked under oxic/anoxic conditions with 10 μl of either 1 mg/ml of HD in methanol or a 1 mg/ml mixture of PDCA, DA and DM in MeOH. The concentration of each analyte in each sediment after spiking was 0.4 μg/g, which corresponds to 2.5 nmol HD/g, 1.8 nmol PDCA/g, 1.5 nmol DA/g and 1.4 nmol DM/g. To get abiotic controls, half of the oxic and anoxic samples were also autoclaved twice to terminate microbial activity before the target CWAs were spiked to the samples. The unspiked control and spiked samples were then incubated in oxic or anoxic conditions for 0 weeks (T0), 1 week (T1), 2 weeks (T2), 4 weeks (T3), 4 months (T4) or 1 year (T5). Of the 276 sediment samples prepared in the WARTOX-project, 144 were selected for this study (Table A.3 in SI). Six of the selected samples were unspiked control samples from JML. These were used to exclude possible false positives rising from the sample preparation and analysis. The 6 control samples from the Bornholm dumping-site were selected to investigate whether the novel analytes are present in CWA-contaminated sediment. The remaining 132 sediment samples selected were Bornholm sediment samples spiked with HD or As-CWAs. These samples were used to study how spiking, microbial activity, oxygen conditions and incubation time affect the measured signal of the novel analytes. A schema of the experimental setting can be found in reference (Rantanen et al., 2024) and in Figure S.4 SI A.

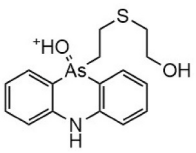
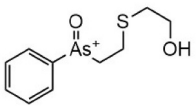
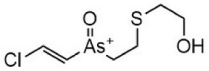
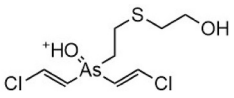
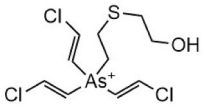
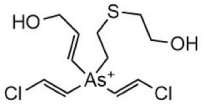
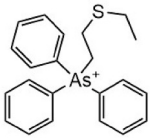
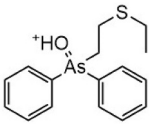
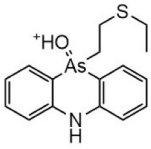
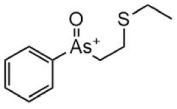
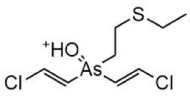
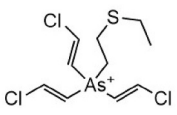
Once the incubation was completed, the sediment portions were centrifuged (5 min, 1000xG) to separate the pore waters and the sediment solid phases. The pore waters and half of the solid phases were conserved for later analysis (not included in this work). The other halves of the solid phases (6 ± 2 g) were extracted twice with 10 ml ACN under vortexing for 10 min. The extracts were combined in volumetric flasks and volumes were adjusted to 20 ml. Half (10 ml) of the extracts were evaporated to dryness in a GeneVac EZ-2 centrifugal evaporator (Genevac Ltd.; Ipswich, United Kingdom). 500 μl of MeOH was added to the evaporation residues and then the samples were vortexed for 10 min. Then 500 μl of ultra-pure water was added to the samples and vortexed for additional 10 min. Before analysis the samples were filtered through syringe driven 0.2 μm Millex®-LG hydrophilic polytetrafluoroethylene (PTFE) membrane filters (Merck; Darmstadt, Germany) into vials. The extracts were analysed by UHPLC-HRMS for transformation products of the studied CWAs ((Rantanen et al., 2024) and unpublished data). In this work the UHPLC-HRMS data was re-analysed for the presence of the novel complexes identified from the reaction mixtures. Since the accurate masses of several of the novel complexes were detected in the extracts, targeted screening and verification of the novel analytes using the developed MRM method and verification analysis by UHPLC-MS/HRMS from selected extracts were also performed. For verification, the fragmentation patterns measured from the samples had to match with the fragmentation patterns of the synthesised reference chemicals and fulfil EU criteria for data interpretation (see section 3 and Table S.26 in SI). A

Table 1
Products of reaction between As–CWAs and HD or CEES detected by UHPLC-HRMS. Mass difference = ((Theoretical m/z –Measured m/z)/Theoretical m/z)•10^6. n.a. = not analysed, n.d. = not detected, n.v. = not verified.

Compound	Elemental composition	Proposed structure of ion	Measured m/z	Theoretical m/z	Mass difference [ppm]	Relative intensity of $[(M + H)+2]^+$-ion [%]	Retention time [min]	Detection in environmental samples
A	$C_{22}H_{24}AsOS^+$		411.07581	411.07583	−0.05645	3.58	2.04	passive sampler, sediment
B	$C_{22}H_{23}AsClS^+$		429.04219	429.04195	0.57582	35.21	2.45	passive sampler
C	$C_{24}H_{27}AsClOS^+$		473.06828	473.06816	0.24065	33.37	2.48	n.v.
D	$C_{24}H_{28}AsOS_2^+$	 	471.07915 471.07913 471.07909	471.07920	−0.11793 −0.15740 −0.24041	7.56 6.20 7.48	2.15 1.61 1.68	passive sampler, sediment
E	$C_{23}H_{26}AsOS^+$		425.09165 425.09138	425.09148	0.39842 −0.24555	3.28 3.66	2.14 2.10	passive sampler, sediment
F	$C_{27}H_{34}AsO_2S_2^+$		529.12073	529.12107	−0.64651	8.01	2.30	passive sampler, sediment
G	$C_{26}H_{32}AsOS_3^+$		531.08243	531.08258	−0.26628	11.39	2.32	passive sampler, sediment
H	$C_{26}H_{32}AsO_2S_2^+$		515.10548	515.10542	0.12083	7.30	2.21	passive sampler, sediment
I	$C_{16}H_{20}AsO_2S^+$		351.03950	351.03945	0.13945	3.97	1.78	passive sampler, sediment

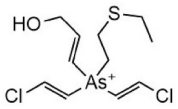
(*continued on next page*)

Table 1 (*continued*)

Compound	Elemental composition	Proposed structure of ion	Measured *m/z*	Theoretical *m/z*	Mass difference [ppm]	Relative intensity of $[(M+H)+2]^+$-ion [%]	Retention time [min]	Detection in environmental samples
J	$C_{16}H_{19}AsNO_2S^+$		364.03452	364.03463	0.30369	3.83	1.52	n.d.
K	$C_{10}H_{14}O_2AsS^+$		272.99244	272.99250	−0.22380	3.13	1.45	n.d.
L	$C_6H_{11}AsClO_2S^+$		256.93790	256.93788	0.10208	35.15	1.10	n.d.
M	$C_8H_{14}AsCl_2O_2S^+$		318.93000	318.93020	−0.65199	68.03	1.46	n.d.
N	$C_{10}H_{15}AsCl_3OS^+$		362.91200	362.91197	0.10425	100	1.50	n.d.
O	$C_{11}H_{18}AsCl_2O_2S^+$		358.96144	358.96150	−0.17853	65.30	1.43	n.d.
P	$C_{22}H_{24}AsS^+$		395.08116	395.08092	0.60526	3.52	2.54	n.a.
Q	$C_{16}H_{20}AsOS^+$		335.04456	335.04453	0.08046	4.36	2.20	n.a.
R	$C_{16}H_{19}AsNOS^+$		348.03983	348.03978	0.12669	3.94	1.98	n.a.
S	$C_{10}H_{14}AsOS^+$		256.99777	256.99758	0.71068	4.44	1.45	n.a.
T	$C_8H_{14}AsCl_2OS^+$		302.93529 302.93523	302.93529	−0.00997 −0.18350	67.00 55.24	1.96 1.86	n.a.
U	$C_{10}H_{15}AsCl_3S^+$		346.91693	346.91705	−0.34518	95.26	2.00	n.a.

(*continued on next page*)

Table 1 (*continued*)

Compound	Elemental composition	Proposed structure of ion	Measured *m/z*	Theoretical *m/z*	Mass difference [ppm]	Relative intensity of $[(M+H)+2]^+$-ion [%]	Retention time [min]	Detection in environmental samples
V	$C_{11}H_{18}AsCl_2OS^+$		342.96649 342.96652	342.96659	−0.30357 −0.19734	63.58 79.64	1.97 1.87	n.a.

schema of the experimental setting and sample preparation can be found in Figure S.4 in SI. A simplified schema of the sample preparation of the sediments and their role in the experimental design of this work is presented in Figure S.2 in SI.

2.4.2. Passive samplers (The Norwegian Defence research Establishment's survey)

The Norwegian Defence Research Establishment (FFI) deployed two types of passive samplers in three locations in Skagerrak. Rig 1 was placed as close to the wreck as possible (only a few meters from the wreck). Rig 2 was located 180 m and Rig 3 7 km from the wreck (Figs. 2 and 4). The passive samplers used were Semipermeable Membrane Devices (SPMD) and Polar Organic Chemical Integrative Samplers (POCIS) from EST-lab.com (delivered by Exposmeter; Tavelsjö, Sweden). One of each sampler type was deployed in Rigs 2 (samples S2-1 and S2-2) and 3 (samples S3-1 and S3-2), and two SPMD and two POCIS samplers were deployed in Rig 1 (samples S1-1, S1-2, S1-3 and S3-4) (Table S.2 in SI). The samplers at Rigs 2 and 3 were allowed to collect material for 10 weeks. Due to unexpected circumstances, the samplers at Rig 1 could not be retrieved at the same time and were left in the sea for 51 weeks. After retrieval from the sampling sites, the passive samples were gently washed with seawater and packed separately in aluminium foil. The samplers were stored at −18 °C prior to sending to VERIFIN for chemical analysis. At VERIFIN, the polymer membrane and the adsorbent of the passive samplers were transferred into 50 ml centrifugal tubes and 25 ml of ACN was added to the tubes. The samples were vortexed for 30 min after which the extracts were filtered (Whatman 5) into 50 ml volumetric flasks. The extraction was repeated with 25 ml ACN and the extracts were combined. The volumes of the extracts were analytically diluted to 50 ml and the extracts were then transferred into 50 ml centrifugal tubes to which approximately 1 g of anhydrous Na_2SO_4 had been added. The extracts were left to dry overnight. 20 ml of the dry extracts were taken for LC-MS analysis. The rest of the extracts were stored for later analysis by GC-based techniques (not included in this work). The 20 ml portions for LC-analysis were evaporated to dryness under mild N_2 flow at 45 °C. 500 μl of MeOH was added to the evaporation residues. The samples were vortexed for 10 min, after which 500 μl of ultra-pure water was added. The samples were vortexed for additional 10 min and then filtered through 0.2 μm Millex®-LG hydrophilic PTFE membrane filters into vials. Unused samplers and a solvent control sample were prepared similarly. The passive sampler extracts and control samples were analysed by UHPLC-HRMS for the identified As-CWA-HD-complexes. Since the accurate mass of many of the novel complexes were detected, the samples were further analysed with the target MRM-method (screening and verification). For verification, selected extracts were also analysed by UHPLC-MS/HRMS to obtain fragmentation patterns of the analytes. The fragmentation patterns measured from the samples were compared with the patterns measured for the synthesised reference chemicals. A list of the samples and a schema of the sample preparation of the passive samplers can be found in Table S.2 and Figure S.3 in SI. A simplified schema of the sample preparation of the passive samplers and their role in the experimental

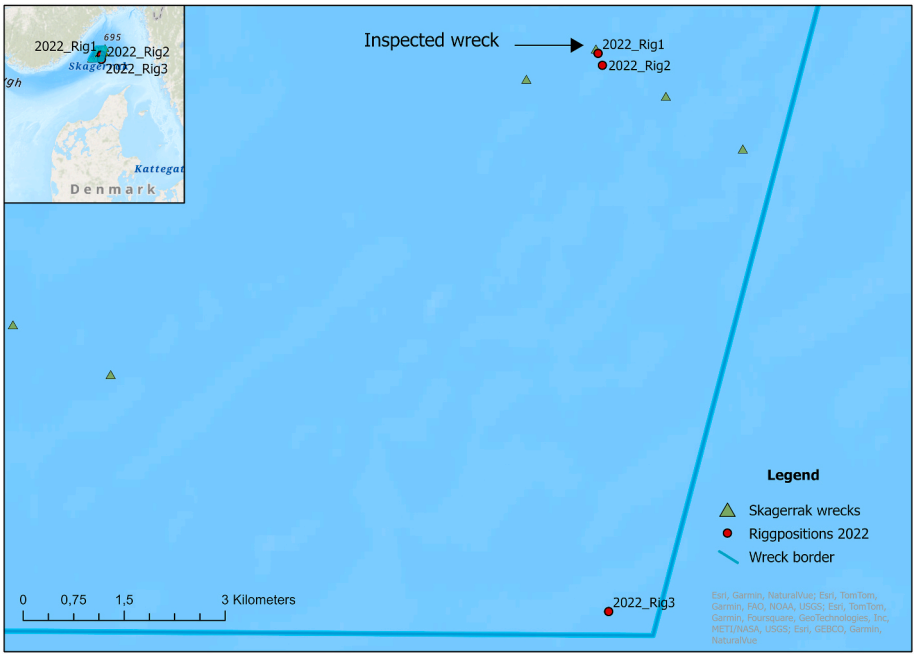


Fig. 2. Map over the sampling sites in Skagerrak. Rig 1 was located only a few meters from the inspected wreck. Rigs 2 and 3 were placed at 180 m and 7 km distance from the wreck, respectively. A closer image of the positions of Rigs 1 and 2 can be found in Fig. 4. Passive samples S1-1, S1-2, S1-3 and S1-4 were deployed on Rig 1, samplers S2-1 and S2-2 on Rig 2, and samplers S3-1 and S3-2 on Rig 3. A map over the sediment sampling sites can be found in reference (Rantanen et al., 2024).

design of this work is presented in Figure S.2 in SI.

3. Results

The total ion chromatograms (TICs) of the reaction mixtures and the crude synthesis product obtained by UHPLC-HRMS analysis were assessed for complex formation between the As-CWAs and HD. Additional analytes were predicted based on the complexes found in the TICs and the known impurities in the L1, L2, and HD stocks, and the masses of the predicted analytes were extracted from the TICs (extracted ion chromatograms, EICs). Interesting ions (i.e. accurate masses and elemental formulas that looked like hypothetic reaction products) found in the TICs and the EICs were further investigated by UHPLC-MS/HRMS analysis using stepped HCD fragmentation. For identification, the measured accurate mass had to be within 5 ppm from the theoretical accurate mass. The $[(M+H)+1]^+$ peak was used to estimate the number of carbon-atoms in the ion, $[(M+H)+2]^+$ peaks were used to evaluate the presence of sulphur and chlorine in the ion, and fragmentation patterns were used to propose structures of the detected analytes. A step-by-step description of the data processing workflow can be found in SI B.

In total, 22 analytes were identified from the reaction mixtures and the crude synthesis product (compounds A–V in Table 1). HD reacted with all As-CWAs (compounds A, B and I–O), but not with the three As-containing degradation products tested (TPAO, DPAA and b-CVAOA). TDG did not react with any of the arsenicals. The HD-stock also contained other longer chained S-mustards, all of which reacted with TPA in a similar manner during the synthesis of compound A (compounds C–H). Though being less reactive than HD, CEES also reacted with all As-CWAs except L1 (compounds P–V). The fragmentation of the analytes suggest that alkylation of the As-atom by the S-mustards had occurred, after which the complex had hydrolysed and oxidised to As(V)-species by water in the reaction mixture. The unbound side of the S-mustard had also hydrolysed in most of the identified analytes. Two half-mustard complexes with chlorine in the unbound side of the mustard (compounds B and C) were found from the crude synthesis product when the sample was analysed soon after the reaction was finished. Nine of the identified analytes were TPA-complexes and most of them were detected in the crude synthesis product. The detected complexes are summarised in Table 1 and their fragmentation patterns are presented in Tables S.4-25 in SI.

The synthesised reference chemicals were used to build an MRM-method, for targeted analysis of the identified complexes from environmental samples by UHPLC-MS/MS. The transitions selected for each compound and their collision energies are compiled in Table S.27 in SI. A quality control sample (approximately 0.01 mg/ml) containing compounds A and D–O was prepared by diluting the reaction mixtures with ultra-pure water. The quality control sample and solvent blanks were analysed regularly in the sequence to monitor instrument performance and to ensure that there is no carry-over between the samples. Chromatograms of the quality control sample are presented in Figure S.5 in SI. Only the hydrolysed complexes between HD and the As-CWAs (compounds A and D–O) were included in the MRM-method because the HD-complexes were considered more relevant than the CEES-complexes. Compounds B and C were excluded because they were no longer present in the reference sample when the MRM-method was created. The retention times, transitions, and collision energies of the analytes included in the MRM-method are listed in Table S.27 in SI. Targeted MRM-analysis revealed that compounds A and D–I are present in the sediments and passive samplers collected from CWA-dumping areas, but not in the uncontaminated control samples, in the unused passive samplers and in the ACN control sample. For verification, the measured ion ratio of two selected ion transitions of the analyte in the sample, had to be within an acceptable range from the ion ratio of the reference chemical. EU criteria for the retention time ($t_{reference}$ - 0.2 min $< t_{sample} < t_{reference} + 0.2$ min) and the tolerance range for the ion ratios were used (Commision, 2002) (Table S.26 in SI). The measured peak areas of compounds A and D–I are tabulated and graphically presented in in Tables S.28 and S.29 and Figures S.6–13 in SI. For further verification, the fragmentation patterns of compounds A, D, E, and H were measured by UHPLC-MS/HRMS and compared with the fragmentation of the reference chemicals (Tables S.4, 7, 8, and 11 in SI). The low concentrations of compounds F, G, and I in the samples did not allow verification by UHPLC-MS/HRMS.

Because the two chlorinated As-CWA-HD-complexes (compounds B and C) were not included in the target MRM-method, we searched for the compounds from the UHPLC-HRMS data of the environmental sample extracts. The accurate masses of both analytes were detected in passive samplers S2-1, S1-1, and S1-2 at retention times that fulfilled the identification criteria. The fragmentation patterns of the ions were measured using UHPLC-MS/HRMS. By comparing the fragmentation patterns of the analyte detected in the sample and the reference chemical, compound B was successfully identified from the passive sampler extracts (Table S.5 in SI). Compound C could not be verified from the passive sampler extracts, because a good spectrum could not be produced due to the low quantity of the analyte in the passive sampler extracts.

4. Discussion

4.1. Structures of the as-CWA-HD- and as-CWA-CEES-complexes

Fifteen As-CWA-HD-complexes were identified from the reaction mixtures of HD and As-CWAs and the crude product (compounds A–O, Table 1). TPA, DA, DC, PDCA, L1, L2, L3, and 3-(bis(-2-chlorovinyl) arsaneyl)prop-2-en-1-ol (L-OH; impurity in the L1 and L2 stocks) (Fig. 1) all produced a product with one hydrolysed mustard tail (*m/z* 105.03686 in Fig. 3) attached to the As-atom (compounds A and I–O). As expected, DA and DC produced the same product in the reactions with HD (compound I), but DA seemed more reactive than DC. This observation can be explained by chlorine having higher electronegativity than carbon and nitrogen, making chloride (Cl^-) a better leaving group than the cyano (CN^-) group. Very low intensities were measured for the PDCA- and L1-complexes, suggesting poor reactivity or inefficient

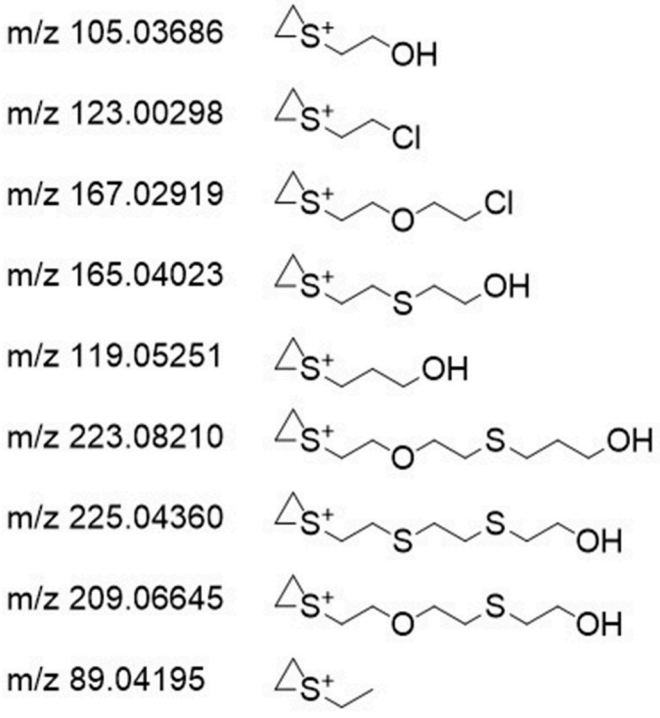


Fig. 3. Accurate masses and structures of the mustard tail fragments.

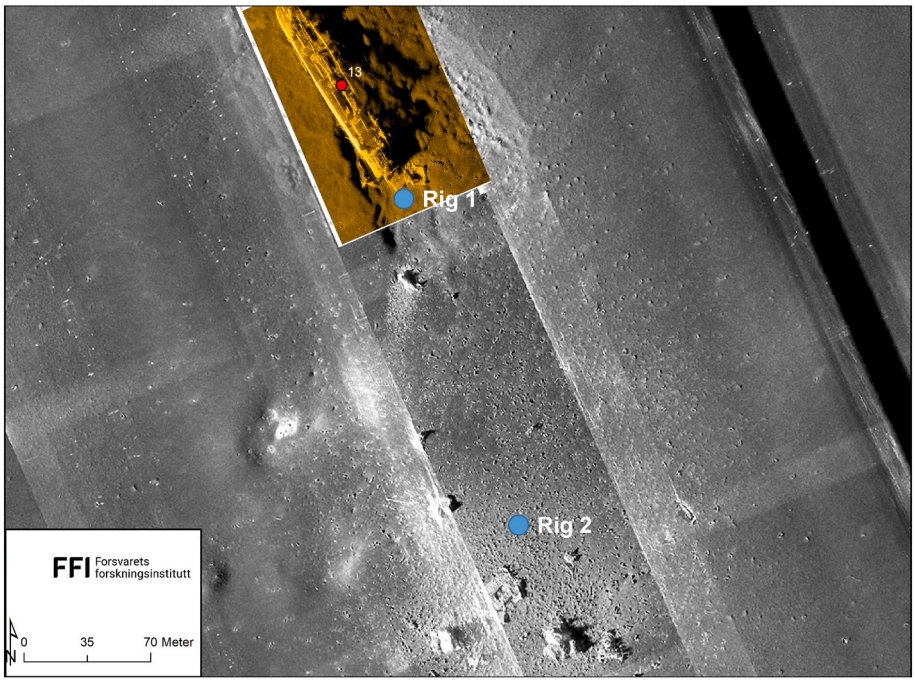


Fig. 4. Synthetic Aperture Sonar (SAS) image of the area around wreck number 13, showing positions of the two rigs with passive samplers (Rig 1 and 2). Rig 1 was located close to the remaining wreck and Rig 2 was located in an area with much ammunition and wreckage 180 m south of the main wreck.

ionisation during analysis. PDCA and L1 are very reactive molecules that produce polymeric structures before oxidation to their primary degradation products. The polymeric structures are quite stable and might therefore limit the reactions with HD. Interestingly, PDCA and L1 produced no complexes with two mustard tails, though they contain two reactive Cl^- substituents on the As-atom. It is probable that the twice-substituted complexes are not formed because of the limiting polymerisation reactions of PDCA, L1 and HD (this section and section 1). From the crude product resulting of the attempt to synthesise compound A, seven additional TPA-HD-complexes were found (compounds B–H). In these complexes a chlorinated and/or longer mustard tail was attached to the As-atom. Compound G (Table 1) was the largest analyte found, with a hydrolysed mustard tail with eight carbons and three sulphur-atoms.

All HD-complexes produced a corresponding mustard tail-ion when fragmented (*m/z* 105.03686, 123.00298, 167.02919, 165.04023, 119.05251, 223.08210, 225.04360, and 209.06645) (Fig. 3), which subsequently produced logical smaller fragments (Tables S.4–18 in SI). Compounds F, and H contained mustard tails with both sulphur and oxygen. The exact position of the sulphur- and oxygen-atoms could not be determined from the fragmentation (Tables S.9 and S.11) so their structures were proposed based on the known impurities in the HD-stock. For the TPA-, L3-, and L–OH–HD-complexes, the As-frame-ion formed after fragmentation of the mustard tail could not be detected. This was expected, as high collision energies are usually needed to fragment TPA, L3, and similar structures. The DA/DC-HD- and DM-HD-complexes produced fragments *m/z* 228.99930 and 241.99455, respectively, corresponding to the phenylarsenic-frame of the As-CWAs (Table B.12 and B.13 in SI). The fragmentation of the HD-complexes of PDCA, L1, and L2 provided evidence of the alkylation of the As-atom (Tables S.14–16 in SI). These analytes produced fragments *m/z* 194.94555, 150.91933, 285.89616, and/or 267.88560, which correspond to an As-atom with a mustard tail and oxygen or vinyl groups attached to it.

Seven analytes were identified from the reaction mixtures of CEES with As-CWAs (compounds P–V, Table 1). These were the CEES-complexes of TPA, DA/DC, DM, PDCA, L2, L3, and L-OH. Similarly to the reactions with HD, the same product was formed in the reaction of CEES with DA and DC (compound Q). Based on the intensities, less of the CEES-complexes were produced compared with the corresponding HD reactions. This was expected, since CEES is less reactive than HD and has only one alkylating functional group. The lower reactivity of CEES resulted in very small quantities of the Lewisite-CEES-complexes (compounds T–V). The only As-CWA that did not react with CEES during the over-night incubation was L1, presumably because the before-mentioned polymerisation of L1 hinders reactions between the CWAs. Typical fragments of the CEES-complexes were *m/z* 89.04195 (Fig. 3) and 61.01065, which are $C_4H_9S^+$ and $C_2H_5S^+$-ions produced from the CEES-tail (Tables S.19–25 in SI). The CEES experiments were useful in the determination of the position of the oxygen-atom(s) in compounds A, N, and O (HD-complexes of TPA, L3 and L-OH). Whether the oxygen-atom (or one of the oxygen-atoms in the case of compound O) is located in the mustard tail or attached to the arsenic-atom could not be determined from the fragmentation of compounds A, N and O. However, the elemental composition of the corresponding CEES-complexes (compounds P, U and V) showed that the complexes contain no (compounds P and U) or only one (compound V) oxygen atom. Since the complexes had the same number of oxygens as the intact CWAs, we concluded that the complexation reaction does not add an oxygen-atom to the As-atom and that the oxygen in compounds A, N and O must to be located in the mustard tail. The fragmentation of the hydrolysed mustard tails (*m/z* 105.03686, 165.04023, 119.05251, 223.08210, 225.04360, and 209.06645 in Fig. 3) supports this hypothesis. Similar conclusions can also be drawn also from compounds B and C, in which the only logical position for the chlorine-atom is in the mustard tail.

On oxidation state (V), As typically forms five bonds. Here all complexes were detected as ions with a positive charge on the As-atom, which are produced in the ion source by the loss of the fifth substituent bound to the As-atom. The fifth substituent could not be identified from the UHPLC-MS/HRMS-data of the HD-and CEES-complexes. Because the reactions and analysis were performed in solutions containing mainly water, the fifth substituent could be an OH-group which is also easily eliminated when the analyte is protonated during the analysis. It is also possible that the complex does not have a fifth

substituent and is instead ionic, with for example Cl^- as a counter ion. Isolating compound A for NMR analysis from the crude synthesis product using different solid phase extraction (SPE) cartridges was attempted. However, the attempts resulted in either insufficient separation of compound A and TPAO (the main by-product of the reactions), or in degradation of compound A during the elution. More experiments are required to isolate compounds A–V, because NMR-data on the complexes would provide additional structural information that will allow verification of the structures proposed in this work.

4.2. Insights on the complexation-reaction

Because of As' high affinity to sulphur, reaction products containing As–S bonds (Tørnes et al., 2006), (Valdez et al., 2018), (Söderström and Östin, 2017), (Sokołowski and Konopski, 2007), (Sokołowski et al., 2008), (Haas, 1998), (Stan'kov et al., 2011) were expected, but instead all As-CWAs were alkylated by the S-mustards. This is the first time alkylation of As-CWAs by S-mustards is reported. The products have presumably formed with a mechanism similar to the known alkylation mechanism of HD (Figure S.1 in SI). The proposed reaction mechanism of for the formation of TPA-HD (compound B) and its hydrolysis to the TPA-TDG-complex (compound A) is presented in Figure S.14 in SI as an example. Both the thiolation/thioesterification of As and alkylation of As happen through nucleophilic substitution reactions. In the thiolation/thioesterification the sulphur-atom with its free electron pairs is the nucleophile attacking the electrophilic As-atom. The alkylation reactions of S-mustards start with an intramolecular nucleophilic substitution reaction that forms the sulphonium-ion (Figure S.1 in SI). As has two electrons in the 4s orbital that form sp^3 hybrid orbitals with the three electrons in the 4p orbitals (Garje and Jain, 1999), (Hirano, 2020). The electron pair in the sp^3 hybridised As-atom can be either non-bonding (as in the thiolation/thioesterification reaction) or bonding, of which the latter makes the As-atom nucleophilic (Garje and Jain, 1999). As, being a nucleophile, can attack the electrophilic sulphonium-ion, forming an As–C bond. However, the nucleophilicity and electrophilicity of organoarsenicals depends on the oxidation state of the As-atom and the substituents. In As(V), the sp^3 hybridisation is extended with a 4d-orbital (in some cases leading to sp^3d hybridisation) and one of the electrons in the free electron pair moves to the d-orbital, allowing As to form five bonds (Garje and Jain, 1999), (Hirano, 2020). Due to their electronic structure, As(V) species are less reactive than As (III) species and usually electrophilic, which may be the reason that no complexes were formed when TPAO, DPAA, or b-CVAOA was used as one of the reactants. Substituents that are very electron withdrawing, like halogens, make both As(III) and As(V) species more electrophilic, while electron donating groups increase the nucleophilicity of As (Garje and Jain, 1999). Other factors, like solvent effects and the strength of the properties of the other reactants, also affect the electrophilicity/nucleophilicity of As. In our experiments, no reaction products with As–S bonds were detected, presumably because the alkylation reaction is faster due to the rapid formation of the electrophilic sulphonium-ion. In addition, the alkyl groups in the S-mustards are poor leaving groups, which makes thioesterification reactions unbeneficial. A more thorough investigation of the mechanism of the formation of compounds A–V would be an interesting research topic for future studies.

To find the best ACN/water ratio for the synthesis, reaction mixtures of TPA and HD in 5%, 25%, 50%, 75% and 100% ACN were prepared. The reaction mixtures, containing approximately 1 ppm of the CWAs, were prepared by spiking 1 μl of the 1 mg/ml CWA stocks into 1 ml of the solvent mixture. The reaction mixtures were vortexed, incubated overnight at room temperature, and analysed with UHPLC-HRMS. The highest signal of compound A was measured in the mixture with 25% ACN/water. Therefore, we chose to use 20–25% ACN in the synthesis. Compound A was also detected in the mixtures containing 5% and 50% ACN/water, but not in the 75% ACN/water and 100% ACN mixtures, which suggested that water is needed for the complexation reaction to happen. The reactivity of HD and CEES is driven by the formation of the sulphonium-ion (Wang et al., 2012), (Bae and Winemiller, 2013), which is formed more efficiently in aqueous solutions than in organic solvents. This is because water can stabilise the positively charged sulphonium-ion. Some polar solvents are also able to stabilise the sulphonium-ion but not as efficiently as water (Wang et al., 2012). For the synthesis of compound A, 20–25% ACN/water is optimal because there is a sufficient amount of water to allow the formation of the sulphonium-ion but enough organic solvent to keep the hydrophobic CWAs solubilised and to slow down the hydrolysis and oxidation reactions that compete with the formation of the complex. The hydrophobicity of HD, CEES and As-CWAs may also be important for the formation of the complexes in aqueous solutions: the hydrophobic CWAs prefer interacting with each other and are therefore not dissolved in the water very efficiently, giving them more time to react with each other to form the complexes.

4.3. Reactivity of degradation products

To investigate if the primary degradation products of the target CWAs of this study can react with each other like their parent compounds, we prepared reaction mixtures of TDG with TPA, DA, L2, TPAO, DPAA and b-CVAOA. No reaction products where the As-atom had been alkylated by TDG were detected. This is not a surprise, because although TDG is nucleophilic it is significantly less reactive than HD. Interestingly, no arsine-ethers that are reported in previous studies were detected either (Valdez et al., 2018), (Sokołowski and Konopski, 2007), (Sokołowski et al., 2008), (Stan'kov et al., 2011), (Epure et al., 2010), (Haas and Krippendorf, 1998), (Opstad, 2015). The unreactiveness of the primary degradation products (TPAO, DPAA, b-CVAOA) is presumably due to the stable hybrid orbital structure of As(V) species described in section 4.2. TPA, on the other hand, is not electrophilic and therefore does not react with the nucleophilic oxygen-atoms. Similar results have also been reported for reactions between L3 (Fig. 1) and alcohols (Valdez et al., 2018). The unreactiveness of DA and L2 was surprising considering that TDG has been successfully used to derivatise Lewisites for GC analysis, and that reaction products of PAO and TDG have been reported (Sokołowski and Konopski, 2007), (Sokołowski et al., 2008), (Opstad, 2015). While many studies have prepared the ether-derivatives by incubating the As–CWAs with TDG or other alcohols at room temperature (Stan'kov et al., 2011), (Epure et al., 2010), (Haas and Krippendorf, 1998), (Opstad, 2015), the best yields have been obtained with incubation at 40 °C (Sokołowski and Konopski, 2007), (Sokołowski et al., 2008). The reaction conditions (temperature, solvent, reaction-time, etc.) of our reaction mixtures may therefore have been unfavourable for the formation of TDG-derivatives of the tested As-CWAs. In addition, GC-based techniques have been used in previous studies to detect As-CWA-TDG-complexes, but here we used LC-MS-based techniques to analyse the reaction mixtures. It is possible that the products formed in the reaction mixtures are not detectable by LC-MS-based techniques. Poor stability of the alcohol derivatives of Lewisites have been reported in previous studies (Sokołowski and Konopski, 2007), (Sokołowski et al., 2008), (Epure et al., 2010), (Haas and Krippendorf, 1998). Possible reaction products may, therefore, already have degraded before analysis.

Because no As-CWA-TDG-complexes where the As-CWA had been alkylated by TDG were detected in the reaction mixtures, and because the previously reported TDG-derivatives have a different structure (arsenic-ethers), we propose that the As-CWA-HD- (compounds A–O) and As-CWA-CEES-complexes (compounds P–V) are formed only in reactions between the intact CWAs, and could therefore be used as indicators of CWA-dumping alongside the other known transformation products. Based on our experiments, we believe that the novel complexes are only formed in munitions containing mixtures of HD and As-CWAs, such as winter-grade HD, and that the occurrence of the

complexes in the environment could provide information on the content of the dumped chemical warfare material. More work is needed to verify this hypothesis and to understand the differences in the reactions of the intact CWAs and their transformation products.

4.4. Occurrence in environmental samples

Environmental samples from two known dumping sites, Bornholm and Skagerrak, were analysed in this study. HD and As-CWAs have been dumped in both dumping sites and their transformation products have been found in both sites (GLASBY, 1997), (HELCOM et al., 2013), (Tørnes et al., 2006), (Söderström et al., 2018), (Bełdowski et al., 2016), (Opstad, 2015). Of the novel analytes, compounds A and D–I were detected in the passive sampler and sediment extracts, which adds eight compounds to the previously reported PAO-complexes found in Skagerrak sediments (Opstad, 2015). Of the novel analytes detected, compound A was the most prominent in all environmental samples (Tables S.28 and S.29 in SI). The analyte signals measured for the passive samplers were generally higher than those of the sediment extracts. Though the analysis does not allow quantitative comparison between the samples, the higher signals suggests that there is more of the novel analytes in the passive sampler extracts than in the sediment extracts. The higher signals measured from the passive samplers could be explained by two factors. Firstly, the passive samplers were used to study the Skagerrak dumping site while the sediments were collected from Bornholm dumping site. More chemical warfare material was submerged in Skagerrak than in Bornholm, which may correlate with the occurrence of the novel analytes (HELCOM et al., 2013), (Tørnes et al., 2020). Secondly, a large volume of water is sampled by the passive samplers, while the sediment sampling provides information on the contamination status of a very small volume of sediment. A larger sampling volume will likely mean that more of the target analyte is collected and thus lead to higher analyte signals (Zalewska et al., 2023). In addition to the differences in the quantity of dumped CW-materials and the sampling methods, the possibility of greater signal suppression due to the more complex matrix in the sediment extracts cannot be excluded. To get more comparable results for the two dumping sites, a new sampling campaign, where the same sampling technique is used for both sites, should be performed. Quantification of compounds A–I and other As-CWA-S-mustard-complexes occurring in the environment should also be considered in future works, as it would be helpful in the assessment of environmental load caused by the submerged chemical warfare material.

As described in section 2.4 and reference (Rantanen et al., 2024), the Bornholm samples consisted of sediments spiked with HD or with As-CWAs (PDCA, DA, and DM), some of which were autoclaved to terminate microbial activity before spiking. The spiked sediments were incubated in oxic or anoxic conditions for 0 weeks to 1 year before analysis. Unspiked control samples of Bornholm sediments were prepared similarly. Compounds A and D–I were detected in the Bornholm sediments (Table S.29 and Figures S.7–13 in SI). The occurrence of the analytes in the unspiked Bornholm sediments shows that the As-CWA-HD-complexes are present in the sediment matrix. Because no As-CWA-HD-complexes were detected in the sediment samples collected from the uncontaminated reference area JML, the occurrence of compounds A and D–I in the Bornholm sediment suggests that the complexes originate from contaminants present only in Bornholm, such as sea-dumped CWAs. There were no differences in the signals measured for compounds A and D–I from the HD- and As-CWA-spiked sediments put through the same treatment. This indicates that the spiking did not affect the amount of the novel analytes in the samples. As can be seen from Figures S.7–13, the oxygen conditions during the incubation had no effect on the occurrence of compounds A and D–I in the sediments. Slightly higher signals were measure for compounds A and I in the autoclaved sediments (Figures S.7 and S.13 in SI), while autoclavation did not affect the signals of compounds D–H in the sediments. A possible reason for this difference is that the autoclavation has caused changes in the matrix that makes detection of compounds A and I easier. Alternatively, microbes still present in the non-autoclaved samples may have degraded compounds A and I, resulting in lower signals. The latter explanation is less likely, considering that no correlation with the incubation time can be observed in the measured signals. In addition, if bacteria were degrading the complexes, differences between the autoclaved and non-autoclaved samples and decreasing time-dependencies would be observed for all compounds (Rantanen et al., 2024).

None of the novel analytes were detected in the extracts of passive samplers deployed in Rig 3 (S3-1 and S3-2), while eight of the analytes (compounds A, B and D–I) were detected in passive samplers S1-1–S1-4, S2-1 and S2-2 deployed in Rigs 1 and 2 (Table S.28 and Figure S.6 in SI). As shown in Fig. 2, Rigs 1 and 2 were located near the inspected wreck, and Rig 3 was positioned 7 km away from the nearest wreck. This suggests that the As-CWA-HD-complexes are more likely to be concentrated near dumped CW-material. Highest intensities for the complexes were measured for sample S2-1. The analyte signals were 20–95% higher than for the other samplers in Rigs 1 and 2 (samples S1-1–S1-4 and S2-2) all of which had analyte signals of similar magnitudes (Table S.28 and Figure S.6 in SI). Sampler S2-1 was deployed with Rig 2, which was positioned further away from the wreck than Rig 1 (Fig. 4). The higher signals of S2-1 indicate that the concentration of the novel analytes is higher further away from the wreck. Closer inspection of the deployment sites showed that Rig 2 was placed in an area were many badly corroded bombs are scattered in the area (Fig. 4). Thus, it can be concluded that sampler S2-1 was placed in a favourable sampling position either near a leaking object, in a position were ocean currents transfer more chemical warfare material to it, or on an optimal distance from a leaking object, allowing the CWAs to mix into the water, hydrolyse and react with each other efficiently. However, it should be noted that the samplers in Rig 1 were deployed for significantly longer time (51 weeks) than the rest of the samplers (10 weeks). Therefore, the samplers in the different rigs are not directly comparable and therefore definite conclusions about the amount of CWA-related material at the sampling sites cannot be drawn. As described before, longer sampling times often lead to higher concentrations (Zalewska et al., 2023), but longer sampling times can also lead to lower yields, due to unpredictable environmental variations (in e.g. leakage rates) combined with analyte degradation or desorption from the adsorbent. A new sampling campaign and a thorough investigation of the leakage rate and ocean currents in Skagerrak may provide an explanation for the higher signals of analytes in sample S2-1, and the differences in the occurrences of the analytes at the different sampling locations.

4.5. Chlorine-containing as-CWA-HD-complexes in Skagerrak samples

One of the chlorinated As-CWA-HD-complexes (compound B) was verified from the extracts of passive samplers S2-1, S1-1, and S1-2. These samplers were positioned near munitions (sample S2-1) and near the wreck (samples S1-1 and S1-2). Our findings are in good agreement with previous studies were chlorinated HD-related chemicals have been found near leaking objects (Söderström et al., 2018), (Hemström et al., 2020). Similarly to the other analytes detected in the passive sampler extracts, the signal intensities suggest that there is more of compound B in sample S2-1 than in the other samplers, which may be due to a favourable sampling position (see section 4.4). It was interesting, however, that the analytes were detected in water-phase (which is sampled by passive samplers), since chlorinated HD-related chemicals have been detected mainly in mustard lumps or heel (Söderström et al., 2018), (Hemström et al., 2020). A possible reason for the detection of the chlorinated compound B could be the continuous leaking of the analyte from the submerged CW-material and reaching the sampler unhydrolysed. Alternatively, the analyte might stay chlorinated in the environment for a longer time than expected. The hydrolysis of HD is considered fast, however, studies have shown that the intact agent can

still be found in the environment for weeks, even years after it was released into the environment (Hemström et al., 2020), (Chmielińska et al., 2019), (Ashmore and Nathanail, 2008), (Czub et al., 2020). The reason for the slow hydrolysis in the environment is HD's poor solubility in water and the passivation of surface of the droplets by polymerisation reactions (Ashmore and Nathanail, 2008), (Huong et al., 2023). The As-CWAs mixed with HD can also slow down the hydrolysis of HD and the chlorinated complexes (Ashmore and Nathanail, 2008). Compound B contains bulky and hydrophobic phenyl-groups. In water this compound might take a 3D-structure where the phenyl-groups shield the chlorinated mustard tail from the surrounding water, thus slowing down the hydrolysis of the mustard tail.

The water in Skagerrak is more saline than the water in Bornholm basin. The difference in water salinity can explain why the chlorinated As-CWA-HD-complexes (compound B and C) were found in Skagerrak but not in Bornholm. The rate of hydrolysis of HD, and therefore also the chlorinated As-CWA-HD-complexes, is determined by how efficiently the sulphonium-ion is formed through the release of the Cl-substituent in the mustard tail (Wang et al., 2012), (Ashmore and Nathanail, 2008), (Bae and Winemiller, 2013). Mechanistic studies of the hydrolysis of CEES (the half-mustard analogue of HD) have shown that the hydrolysis is slower in solvents containing high concentrations of Cl^--ions (Bae and Winemiller, 2013). Based on the findings, a first-order nature with dependency of the Cl^-- concentration in the solvent has been proposed for the hydrolysis. This together with the knowledge on the salinity of the studied dumping sites, suggests that hydrolysis of HD, other S-mustards, and compounds B and C could be slower in Skagerrak, which explains the detection of compound B in the passive sampler extracts. The Cl^--dependency of the hydrolysis also suggests that more chlorinated HD-related chemicals could also be detected in the Bornholm dumping site when major Baltic inflow brings saline and oxygen-rich water to the Baltic Sea. Sea-water is also rich in radicals, such as chlorine and hydroxyl radicals (Xu et al., 2007), (Lei et al., 2021), (Xie et al., 2022). Chlorine radicals are known to react with organic molecules to produce chlorinated organic compounds (Lei et al., 2021). These chlorination reactions might also contribute to the occurrence of chlorine-containing analogues of the complexes. Hydroxyl radicals similarly add hydroxyl-substituents to organic compounds, but they also affect the oxidation state of arsenic (Xu et al., 2007), (Xie et al., 2022). As mentioned in section 4.2, As can act as a nucleophile when it has the oxidation state III, which is an un-typical oxidation state for arsenic in oxygen rich conditions. Therefore, radicals in the sea-water might play a role in the formation of the novel complexes by reducing CWA-related As(V)-species to As(III)-species, which can then react as nucleophiles with S-mustards in the near surroundings. More research is needed to determine the role of radicals in the formation of the novel complexes and in the transformation of CWAs in marine environments.

4.6. Environmental threat of sea-dumped CWAs and the novel analytes

As can be seen from the structures presented in Table 1, the novel As-CWA-HD- and As-CWA-CEES-complexes contain structural moieties of both As-CWAs and HD. Since As-CWAs and HD produce their toxic effects through different mechanisms, the structural similarities with both CWA-types raises questions about the potential toxic properties of the complexes and how they behave in biological systems.

As-CWAs produce their toxic effect by binding to thiols, therefore causing structural changes in proteins and enzymes, which disturb biological processes (Muzaffar et al., 2023), (Hirano, 2020), (Noort et al., 2002). The size and structure of As and phosphorus (P) are also similar, which allows As to replace P in phosphates in, for example, energy metabolism (Muzaffar et al., 2023). Whether As with organic substituents (for example As-CWAs) can replace P in phosphate has not been studied. Neurological symptoms, brain atrophy, and abnormal development has also been reported as symptoms by victims of As-CWA exposure, however the exact mechanism of how the toxic response is produced has not been determined (Ishii et al., 2004), (Muzaffar et al., 2023), (Isono et al., 2018). Despite extensive studies throughout the years, the mechanism of HD toxicity is still not fully understood (Della Torre et al., 2013), (Tsoutsoulopoulos et al., 2020). It is assumed to be based on HD's ability to alkylate almost any nucleophile it comes across. HD is known to alkylate biomolecules, such as DNA, RNA and proteins, and because it has two reactive sites, it can also crosslink proteins and DNA (Vanninen et al., 2023), (Della Torre et al., 2013), (Noort et al., 2002), (Shakarjian et al., 2010). Alkylation of proteins, DNA and other biomolecules disrupt their normal functions and can lead to cell death. Because the As-CWA-HD-complexes have structural similarities with both As-CWAs and HD, they might have toxic properties of both CWA-types: they might bind to thiols through the As-atom and alkylate biomolecules through the mustard tail. As described in sections 4.2 and 4.4., HD becomes significantly less reactive once hydrolysed. Thus, alkylating properties may be expected only for the chlorinated As-CWA-HD-complexes, such as compounds B and C, though the bulky As-CWA-substituents might limit their reactivity with nucleophiles. Although the hydrolysed mustard tails in the As-CWA-HD-complexes are not alkylating, they might contribute to the toxicity by making the complexes more lipophilic, which allows the complexes to enter cells and to bioaccumulate more efficiently. Knowing that HD was often mixed with As-CWAs, it is possible that some of the symptoms observed in victims of accidental CWA exposure may have been produced by the As-CWA-HD-complexes identified in this work or their chlorinated analogues (Radke et al., 2014), (Söderström et al., 2018), (Chmielińska et al., 2019), (Epure et al., 2010), (Muzaffar et al., 2023), (Isono et al., 2018).

For accurate assessment of the environmental threat of sea-dumped CWAs, knowledge on their quantity, toxicity and accumulation in marine species is needed. Only a few studies have been conducted to determine the toxicity of HD and As-CWAs to marine species (Fauser et al., 2023), (Barbosa et al., 2023). The acute toxicity of As-CWAs and their primary degradation products has been tested with water-fleas and zebrafish as model species (Czub et al., 2021), (Wilczynski et al., 2023), (Wilczynski et al., 2024). These tests have shown that the intact CWAs, excluding TPA, are toxic or very toxic to the test species. No acute toxicity was observed for the primary degradation products. Acute toxicity was observed in rainbow trout liver cells exposed to DPAA and the glutathione-adduct of DA (DA-SG). In the toxicity assay used in the study, DA-SG was one hundred times more toxic than DPAA, which shows that organisms can metabolise CWAs to more toxic compounds (Niemikoski et al., 2021). This is in agreement with previous studies on DA-SGs toxicity to human cells (Ochi et al., 2004). A few studies on HDs toxicity to marine species have been performed mainly using water-fleas and zebrafish as model species (Chmielińska et al., 2019), (Czub et al., 2020), (Huong et al., 2023). Recent studies have shifted their focus to determining the toxicity of HDs transformation products to water-fleas and human cells (Hemström et al., 2020), (Chmielińska et al., 2019), (Czub et al., 2020), (Huong et al., 2023), (Tsoutsoulopoulos et al., 2020). Of all the transformation products tested, TDGS, 2-chlorovinyl sulphone (DVS), 1,4-dithiane, 1,4-oxathiane, 1,4,5-oxadithiane, and 1,4,5-trithiepane were found to be toxic or harmful. TDGS, DVS and 1,4,5-trithiephane were, in fact, found to be more toxic than HD. Since both As-CWAs, HD and related chemicals are toxic to aquatic life (and humans) it is not far-fetched to assume that the As-CWA-HD-complexes are toxic as well. From the findings of previous and this work, it is clear that thorough toxicological investigations of the novel compounds identified in this study, as well as intact CWAs and their transformation products, should be conducted.

Shrimp, eel and fish caught from dumping sites in Hawaii and the Mediterranean Sea have been analysed for HD and its transformation products (Della Torre et al., 2013), (Della Torre et al., 2010), (Kehe et al., 2009). No HD-related chemicals were detected in the species, however, health impairments, such as lesions and DNA-damage were observed in the fish and eel caught from the Mediterranean Sea (Della

Torre et al., 2013), (Della Torre et al., 2010). Sublethal effects (for example geno- and cytotoxicity and lesions) of CWA-exposure have also been reported for fish caught in Skagerrak and in the Baltic Sea, and in blue mussels and zebra fish exposed to CWAs in laboratory experiments (Höher et al., 2019), (Wilczynski et al., 2024), (Straumer et al., 2020), (Pažusienė et al., 2021), (Lastumäki et al., 2020). Analysis of As from the tissues of biota samples caught from Hawaii, Skagerrak and the Baltic Sea have revealed that individuals from dumping sites contain more As than individuals caught from uncontaminated reference sites (Koide et al., 2016), (Della Torre et al., 2010), (Polak-Juszczak and Szlinder Richert, 2021). Speciation analysis of the As in Baltic Sea fish showed that inorganic As and non-toxic metabolites like arsenobetaine are the most prevalent forms of As (Polak-Juszczak and Szlinder Richert, 2021). Whether the As detected in the tissues of marine biota originated from the As-CWAs, the corroded containers or some other non-CWA-related source could not be determined (Koide et al., 2016), (Della Torre et al., 2010), (Polak-Juszczak and Szlinder Richert, 2021). Blue mussels have been exposed to DA and DM in laboratory experiments to study the bioaccumulation of the As–CWAs. Neither DA or DM were detected in the mussels, however, significant amounts of their primary degradation products (DPAA and DM[ox], respectively) were found in the exposed individuals. Similarly, DM-exposure lead to accumulation of DM[ox] in muscle tissue of zebra fish (Wilczynski et al., 2024). DPAA and TPAO have also been detected in the tissues of marine biota caught from Skagerrak and the Baltic Sea using a targeted LC-MS/MS method (Niemikoski et al., 2017a), (Niemikoski et al., 2020c), (Niemikoski, 2022). The bioaccumulation of DA in Baltic Sea food web has been investigated in a recent modelling study (Czub et al., 2024). The modelling showed that DA accumulates more strongly in bottom feeding species, which frequently come in contact with the CWA-contaminated sediment. The study also revealed that commercial fishing and climate change affect the bioaccumulation of DA by changing the demographics and behaviour of the fish population and leakage rate, respectively. These results show that CWAs can bioaccumulate in marine species. The eight As-CWA-HD-complexes (compounds A, B, D–I) that were detected in the passive samplers and sediments have structural similarities with DPAA and TPAO, which have been found in marine biota. Similarly, As-CWA-HD-complexes could putatively bioaccumulate in exposed species. Though PDCA-, Lewisite- and HD-related chemicals have not been found in marine species, the lipophilicity (HD) and high reactivity (PDCA, Lewisites, HD) of these chemicals suggests that they can also accumulate in biota as free metabolites or as adducts (Ashmore and Nathanail, 2008), (Vanninen et al., 2023), (Höher et al., 2019), (Della Torre et al., 2013), (Huong et al., 2023), (Noort et al., 2002), (Shakarjian et al., 2010). Based on this we propose that the As-CWA-HD-complexes identified in this work can form adducts with biomolecules, and that both As-CWA-HD- and As-CWA-CEES-complexes can accumulate in tissues of marine species (and humans) exposed to the complexes. These hypotheses should be investigated in future studies.

From the results described above, it is evident that sea-dumped CWAs are a threat to marine species and ecosystems, and therefore also to humans who utilise the ecosystems. In addition to dumping sites at sea there are also burial, manufacturing and storage sites on land that have been contaminated with CWA (Chmielińska et al., 2019), (Ashmore and Nathanail, 2008), (Ishii et al., 2004), (Muzaffar et al., 2023). These pose a more urgent threat to humans, because they are more easily disrupted and thus more likely to cause accidental exposure and spreading of the CWA-contamination (Ishii et al., 2004), (Muzaffar et al., 2023), (Isono et al., 2018), (Ochi et al., 2004). Two examples of accidents like this are the contamination of well-water by phenylarsenicals in Kamisu, Japan, and the discovery chemical warfare material filled with mixtures of HD and Lewisites in Qiqihar, China (Ishii et al., 2004), (Isono et al., 2018), (Ochi et al., 2004). In both cases, serious and long term negative effects on the victims health and deaths, were reported (Ishii et al., 2004), (Isono et al., 2018). These cases highlight the importance of CWA research. More scientific knowledge on the quantity, bioaccumulation and toxicity of CWAs, their transformation products, and the products formed when the CWAs react with each other or the additives in the munitions, is needed for accurate estimation of the threat of CWAs in the environment.

In addition to raising questions about the toxicity and accumulation of the novel complexes, our findings bring new insight to the reactivity in the environment and in laboratory conditions. This work also provides an additional explanation to why HD-related chemicals are detected not as frequently and in lower quantities than expected based on the recorded dumping practices. It is interesting that the As-CWA-HD-complexes were found in the Baltic Sea region even though the dumping of the CW-material happened decades ago. The occurrence of the analytes in the environment suggests that they are continuously formed and/or leaking from submerged munitions, or are persistent in the environment. Both options are plausible because surveys have shown that the submerged chemical warfare material is badly corroded and that the CWA-related chemicals transform non-toxic chemicals very slowly (Tørnes et al., 2020), (Zalewska et al., 2023), (Czub et al., 2024), (Barbosa et al., 2023), (Höher et al., 2019), (Czub et al., 2021), (Wilczynski et al., 2024). Based on some estimations, the corrosion of the chemical warfare material will escalate in the coming decades, which will lead to increased leaking and environmental stress (Czub et al., 2020), (Czub et al., 2024). Therefore, environmental risk assessments should be done without delay so that remediation projects can be started before the chemicals leak into the environment. This would be of highest importance if the As-CWA-HD-complexes turn out to be as toxic as or more toxic than their parent CWAs.

5. Conclusions

Large quantities of CWAs were submerged in the Baltic Sea region in the aftermath of the Second World War. Although sea-dumped CWAs have been studied in various projects during the last two decades, the extent of the environmental threat they pose is still unclear. In this study we aimed to deepen the understanding of the reactivity of the two CWA-types mainly dumped in the Baltic Sea region: HD and As-CWAs (PDCA, DA, DC, DM, TPA, L1, L2, L3, and L-OH). These CWAs were often used (and therefore also dumped) as mixtures, but studies on the reactivity between these CWAs are scarce. This work is one of the first to investigate the potential reactivity of HD with As-CWAs and the occurrence of the reaction products in the environment. In addition to producing new knowledge on the reactivity of CWAs, this study aimed to investigate whether reactions between the CWAs can provide an explanation to why HD and its transformation products are detected less frequently and in smaller quantities than expected.

Using simple reaction mixtures and UHPLC-HRMS and UHPLC-MS/HRMS analysis we were able to identify 22 novel As-CWA-HD- and As-CWA-CEES-complexes formed in the reactions between HD or CEES with As-CWAs. This is the first time that alkylation of As-CWAs by HD and CEES is reported. The synthesised reference chemicals were used to develop an MRM-method for targeted analysis of the novel analytes. Using the developed method and UHPLC-(MS/)HRMS analysis, eight of the novel As-CWA-HD-complexes were found in extracts of passive samplers and sediments collected from known CWA dumping areas in the Baltic Sea region. Our results show that CWAs can react with each other in laboratory conditions and in the environment. These reactions bring new insight to the reactivity of the target CWAs and they can partially explain the low detection rates and quantities of HD-related chemicals. The occurrence of the As-CWA-HD-complexes in the environment still decades after the dumping, suggests that the novel analytes are, just like their parent CWAs, persistent in the environment.

Information on the quantity, toxicity and environmental behaviour of CWAs and their transformation products are needed for the accurate estimation of the sea-dumped chemical warfare material. Future works should optimise the synthesis and develop a method for the purification of the novel complexes. The purified chemicals would allow

determination of the full structure of the complexes using NMR-analysis, and quantification from environmental samples. The pure reference chemicals could also be used to determine the toxicity, metabolism, accumulation and sublethal effects of the As-CWA-HD-complexes in marine biota. Studies have shown that both the intact CWAs and their degradation products are toxic to aquatic life and cause sublethal effects in marine species. Therefore, it is probable that the As-CWA-HD-complexes identified in this work are also hazardous for marine species and humans. This work shows that more research on the reactivity of HD with other CWAs and chemicals added to the munition is needed and that the products formed in the reactions must be considered when assessing the risks of CWAs dumped in the environment.

CRediT authorship contribution statement

Noora-Kaisa Rantanen: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Marita Ljønes:** Writing – review & editing, Resources, Methodology, Investigation. **Johannes S. Heikkinen:** Writing – original draft, Resources, Methodology. **John A. Tørnes:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Matti A. Kjellberg:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Hanna Hakulinen:** Writing – review & editing, Resources, Funding acquisition.

Declaration of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143575.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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Glossary

(H)ESI: (Heated) electrospray ionisation
ACN: Acetonitrile
As-CWA: Arsenic-containing CWA
b-CVAOA: Bis(2-chlorovinyl)arsinic acid
BSTFA: N,O-bis(trimethylsilyl)trifluoroacetamide
CEES: 2-Chloroethyl ethyl sulphide
CVAOA: 2-Chlorovinylarsinic acid
CW: Chemical weapon
CWA: Chemical warfare agent
DA: Clark I, Diphenylchloroarsine
DC: Clark II, Diphenylcyanoarsine
DM: Adamsite, 10-chloro-5,10-dihydrophenarsazine
DM[ox]: 10-Hydroxy-5H-phenarsazine 10-oxide
DPAA: Diphenylarsinic acid
DVS: 2-Chlorovinyl sulphone
HD: Sulphur mustard
FA: Formic acid
EIC: Extracted ion chromatogram
FFI: Norwegian Defense Research Establishment
GC: Gas chromatography
HCD: High-energy collisional dissociation
HPLC: High-performance liquid chromatography
HRMS: High-resolution mass spectrometry
L1: 2-chlorovinylarsine dichloride
L2: Bis(2-chlorovinyl)arsine chloride
L3: Tris(2-chlorovinyl)arsine
L-OH: 3-(Bis(-2-chlorovinyl)arsaneyl)prop-2-en-1-ol
LC: Liquid chromatography
MeOH: Methanol
MRM: Multi reaction monitoring
MS: Mass spectrometry
MS/(HR)MS: Tandem (high-resolution) mass spectrometry

MTBSTFA: N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide
NMR: Nuclear magnetic resonance spectroscopy
SAS: Synthetic aperture sonar
SPE: Solid phase extraction
SPMD: Semipermeable Membrane Devices
t-CVAOA: Tris(2-chlorovinyl)arsine oxide
TDG: Thiodiglycol
TDGO: Thiodiglygol sulphoxide
TDGS: Thioglycol sulphone
TIC: Total ion chromatogram
PAA: Phenylarsinic acid
PDCA: Phenyldichloroarsine
POCIS: Polar Organic Chemical Integrative Samplers
PTFE: Polytetrafluoroethylene
TPA: Triphenylarsine
TPAO: Triphenylarsine oxide
UHPLC: Ultra-high-performance liquid chromatography
VERIFIN: Finnish Institute for Verification of the Chemical Weapons Convention