# Synthesizing the impact of sea-dumped munition and related chemicals on humans and the environment

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**Abstract**

Marine environments are globally impacted by vast quantities of munition disposed following both World Wars. Dumped munitions contain conventional explosives, chemicals warfare agents as well as a variety of metals. Field monitoring studies around marine dumpsites report the presence of munition constituents in water and sediment samples. The growing interest and developments in the ocean as a new economic frontier underline the need to remediate existing dumpsites. Here, we provide a comprehensive assessment of the magnitude and potential risks associated with marine munition dumpsites. An overview of the global distribution of dumpsites identifying the most impacted areas is provided, followed by the currently available data on the detection of munition constituents in environmental samples and evidence of their toxic potential to human and environmental health. Finally, existing data gaps are identified and future research needs promoting better understanding of the impact of the dumped material on the marine environment suggested.

## 1. Introduction

For centuries, explosives and chemical warfare agents have been used in wars, conflicts and extremist activities. Further, while the first forms of such munition were made of natural resources, the rapid scientific advances observed during the 19th century tremendously increased their potency and efficacy (Johnson, Larsen and Meek, 2015; Chatterjee *et al.*, 2017). Massively produced and used in both World Wars (World War I and World War II), during the early 20th century, the decades following these historical events were marked by the disposal of colossal amounts of munition in aquatic environments (Tørnes *et al.*, 2002). The dumped material consisted mainly of munitions, such as artillery shells, mortar rounds and aerial bombs, and chemicals stored in large metal containers (Tørnes *et al.*, 2002). These were disposed of either unfettered, promoting their dispersal by currents and tides, or loaded as cargo onto ships to be sunk, hence confined to a small area (Tørnes *et al.*, 2002). Marine coastal areas are particularly impacted by relic munition with dumpsites being found all over the world (Brewer and Nakayama, 2008). However, because of the scarcity and/or confidentiality of the records of dumping activities, the total quantity of munitions discarded in the ocean, as well as its distribution, is largely unknown (Sanderson *et al.*, 2017). Regardless of its long historical background, little information is available on the impact of dumped conventional explosives, chemical warfare agents (CWAs) and munition components on human health and the marine environment, even though some of the compounds have been shown to have cytotoxic, genotoxic and carcinogenic effects (Tornero and Hanke, 2016; Sanderson *et al.*, 2017). In fact, risk assessment and environmental toxicology have thus far mainly focused on fish (della Torre *et al.*, 2010; Baršiene *et al.*, 2014, 2016; Sanderson *et al.*, 2014). Moreover, the prolonged exposure of munition to seawater may not only increase the sensitivity of explosive material to detonate, as a consequence of the deterioration of the stabilizing compounds or recrystallization (Pfeiffer, 2012), but also corrode the munition shells causing breaches that allow the release of

toxic chemicals to the environment (Bełdowski, Klusek, *et al.*, 2016; Edwards *et al.*, 2016; Silva and Chock, 2016; Jurczak and Fabisiak, 2017).

Indeed, dumped munition not only contains explosives and toxic agents but may also be a source of heavy metals and metalloids in the environment (Tchounwou *et al.*, 2012; Bełdowski, Szubska, *et al.*, 2016). Corrosion of munition shells in the marine environment is dependent on various factors including the period of exposure to seawater, the type, quality and thickness of the materials constituting the munition, the availability of dissolved oxygen, salinity, temperature, pressure, seafloor current velocity, degree of burial in seafloor sediment and sediment composition, which impacts chemical adsorption potential. Furthermore, the existence and condition of possible protective coatings, e.g. paint, and microbiological processes can also greatly influence the rate of corrosion (HELCOM, 1994; MacLeod, 2016; Jurczak and Fabisiak, 2017). Contrary to what is demonstrated for terrestrial military ranges, where clear metal contamination from weapon use is observed (Stauffer, Pignolet and Corcho Alvarado, 2017), the same definitive link has not thus far been established for submerged munition (Beck *et al.*, 2018). Despite the yet inconclusive link to munition contamination, several studies report the detection of different heavy metals, specifically arsenic, lead, chromium, nickel, zinc and manganese, in the vicinity of various dumpsites in Europe (Valkovic *et al.*, 2009; della Torre *et al.*, 2010; Bełdowski, Szubska, *et al.*, 2016; Gębka, Bełdowski and Bełdowska, 2016; Czub *et al.*, 2017; Bełdowski *et al.*, 2019), North and South America (Biester *et al.*, 2002; Schuster *et al.*, 2002; Ampleman *et al.*, 2004; Corella *et al.*, 2017) and Asia (Sun *et al.*, 2016). The detection of such compounds is in line with various field studies reporting a high degree of corrosion in munition dumped after WWII, as observed over the Hawaiian coast (Silva and Chock, 2016), in the Bornholm Basin (Sanderson and Fauser, 2015) and in the Adriatic Sea (Amato *et al.*, 2006). Moreover, heavy metals have been shown to exert adverse effects on human health and the environment (Tchounwou *et al.*, 2012). While nickel,

zinc and manganese are essential metals, i.e. required for a multitude of biochemical and physiological functions, at high concentrations they may lead to cellular and tissue damage resulting in various deficiency diseases and syndromes (Tchounwou *et al.*, 2012). Arsenic, chromium and lead, on the other hand, are considered systemic toxicants with genotoxic (Jomova *et al.*, 2011; Wani, Ara and Usmani, 2015) and carcinogenic potential (DesMarias and Costa, 2019) also known to impair intellectual and physical development in children and reproductive impairment, brain and kidney damage in adults (Apostoli *et al.*, 1998; Kaul *et al.*, 1999; Hertz-Picciotto, 2000). Further, both essential and non-essential heavy metals have been widely studied for their toxicity to aquatic organisms, bioaccumulation and bio-magnification in aquatic food chains (Tao *et al.*, 2012).

Considering the above mentioned knowledge gaps and potential threats, there is a growing interest in the remediation of undersea munitions in line with a growing recognition of the ocean as a new economic frontier (Appleyard, 2015; Sanderson and Fauser, 2015; OECD, 2016). Joffray et al. state that the current growth of the blue economy is related to three distinct fundamental human needs: food, material and space (Jouffray *et al.*, 2020). In addition to the millennial source of food, via animals and plants, for coastal communities (Jackson *et al.*, 2001), the oceans provide a vast range of resources, such as gas, oil and minerals (Chong *et al.*, 2016; Miller *et al.*, 2018). Further, the claim of these resources require space in the ocean for the development of the necessary infrastructures (Jouffray *et al.*, 2020). Due to the growing use of the ocean, marine ecosystems face unprecedented cumulative pressures. The worldwide scattered, thus far undisturbed, dumpsites will likely be increasingly disturbed, with unclear consequences to human and environmental health (Pfeiffer, 2012; Edwards *et al.*, 2016; Silva and Chock, 2016; Maser and Strehse, 2020, 2021). With this in mind, available literature on the topic was searched using keywords such as “munition dumpsites”, “conventional explosives” and “chemical warfare agents”. For final inclusion in the review, articles were screened for

either (1) first description of the site/effect (milestone) as well as (2) most up-to-date articles addressing historical information on dumpsite distribution, detection of munition-related chemicals in environmental samples and biota, used analytical methods, and effects on human and environmental health. Hence, when multiple articles reporting concentrations on the same munition dumpsite for the same compounds under the same conditions were retrieved, the most recent ones were reported to provide the most recent environmental concentrations. This allows to comprehensively synthese the current knowledge on the occurrence, fate and adverse effects of conventional explosives, CWAs and munition structural components. The global distribution of dumping-sites is first presented allowing the identification of the more severely impacted areas. Munition-related chemical contaminants commonly detected in the environment are subsequently identified, as well as the most relevant physicochemical properties of these compounds and fate processes may be undergo. Finally, knowledge on adverse effects of the previously identified chemicals is summarized thus providing a clear overview of the potential impact of the dumped material on human health and the environment.

## 2. Global distribution

After World War I and World War II, and until the promulgation of the Convention on the Prevention of Marine Pollution by Dumping of Wastes (Oslo Convention) and the US Marine Protection, Research, and Sanctuaries Act, both in 1972, intentional disposal of unusable or unwanted munitions was mainly done in the ocean (discarded munition material, DMM) (Edwards and Bełdowski, 2016; Greenberg, Sexton and Vearrier, 2016). Dumping operations were performed by a total of 40 countries yet mostly conducted by USA, France, Great Britain Japan and Russia (Carton and Jagusiewicz, 2009). In the US such operations started in 1919 (as a result of the end of the 1st World War) and were carried following regulations: 1) dumping was required to take place in marine waters, 2) at a depth of no less than 30 m and 3) at a

distance of at least 65 km from the shore. However, many other countries did not implement similar regulations, hence leading to munition dumping in coastal waters, often in shallow places, and even in freshwater environments such as rivers and lakes (Bełdowski, Been and Turmur, 2017). Additionally, tons of unexploded ordnance (UXO), armed and deployed but undetonated mines, failed detonations or wrecks of ships carrying munition, were dumped in coastal marine environments (Aker, Howard and Reid, 2012). Further, it is has been estimated that 20-30% of the ammunition fired during both world wars failed to detonate (GICHD, 2016). Consequently, DMM and UXO are now found in coastal waters in the Atlantic, Pacific and Indian Oceans, east and west coast of Canada and USA, in the Gulf of Mexico, Australian, New Zealand, India, Philippines, Japan, Great Britain and Irish coasts and the Caribbean, Black, Red, Baltic, Mediterranean and North Seas. African and South American coasts are relatively unimpacted (Brewer and Nakayama, 2008; Bełdowski, Been and Turmur, 2017). In general the location and extent of marine DMM and UXO are largely undocumented. In fact, while the records of some dumping operations are rather detailed, including listings of dumping locations as well as type and quantities of dumped munitions, others were done haphazardly with no or minimal preserved records. In Europe, identified dumpsites exist in the North Sea, i.e. along the coasts of Belgium, Netherlands and Germany, Irish Sea, Biscay Bay and Adriatic and Baltic Seas. These dumped material mainly consists of Mustard Gas, smaller quantities of arsenic agents, such as Clark I and II, Adamsite and Lewisite, together with conventional explosives (Bełdowski, Been and Turmur, 2017). In the Baltic Sea, a total of approximately 50,000 tons of chemicals weapons of German origin were dumped in multiple sites located in Danish, Swedish and German exclusive economic zones, both during and after WW II. Specifically, in the course of the conflict, dumping operations were performed by Third Reich authorities in the area of the Little Belt. Further, in immediate years following the conflict, from 1945 to 1947, the main dumping operations near the Bornholm island and in the Gotland basin were conducted

by Russian occupational forces. Finally, in the decade of 1950, under the command of the German Democratic Republic, dispersed dumping occurred in the southern area of the Baltic Sea. Similarly, following British and American occupation authorities' orders, 168,000 tons of chemical weapons were disposed of in Skagerrak, mainly in the Norwegian trench and off the Swedish coast (Nawała *et al.*, 2016). In other areas of the world, 5,000 tons of munition are known to have been disposed of at seven different locations off the east coast of Japan and approximately 150,000 tons in the White, Barents and Kara seas of the Russian Artic (Brewer and Nakayama, 2008). Less precise information also indicates the existence of 32 disposal sites off the US shores, their composition and extent is, however, poorly documented. The Adriatic Sea is also known to be highly impacted by sunken ships containing munition off the harbors of Bari and Molfetta, Italy (Brewer and Nakayama, 2008; Christensen *et al.*, 2016). Many other similar sites around the globe are suspected to occur. In total, 127 dumping areas are documented while over 300 are suspected to exist (Bełdowski, Been and Turmur, 2017).

While only considering CWAs, The James Martin Center for Nonproliferation Studies developed an interactive map compiling valuable information on various dumpsites providing information on the confirmation status, year of dumping, amount dumped and depth of the dumpsite (https://www.google.com/maps/d/u/0/viewer?ll=5.368292378570278%2C0&z=2&mid=1ALnyOrN5JQ8H50znwJqI_Sj8IwE). Since CWAs were usually dumped in association with conventional explosives, and more detailed information is available with regards to dumping activities of the first (Krohn, 1994), this interactive map can be considered as a reliable source for the identification of the more impacted areas.

Currently, different international organizations have addressed the issues of conventional and chemical munitions dumped at sea. Specifically, the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Convention) as well as the Working

Group on Dumped Chemical Munitions of the Baltic Marine Environment Protection Commission (HELCOM CHEMU) produced comprehensive guidelines for the management of chemical weapons disposed in European waters (OSPAR, 2010; HELCOM, 2013).

## 3. Detection of munition constituents in the environment

Constituents of both conventional and chemical munitions leak to the surrounding environment, i.e. the water column and/or sediment, via breaches in munition shells caused by continued aging and corrosion. Different analytical methods varying in their specificity and detection limits have been reported for the detection and identification of munition constituents in environmental samples (Bromage *et al.*, 2007; Badjagbo and Sauvé, 2012; Xu *et al.*, 2014; Rapp-Wright *et al.*, 2017). A commonly used method relies on solvent extraction followed by separation by high performance liquid chromatography (HPLC) and ultra-violet-visible spectroscopy (UV-VIS) detection, achieving a detection limit in the range of µg/L for nitroaromatic and nitramine compounds (US EPA, 2007). However, this approach suffers from some limitations as UV-VIS does not allow the detection of poor light-absorbing munition constituents like nitroglycerine and [3-Nitrooxy-2,2-bis(nitroxymethyl)propyl] nitrate (PETN). Furthermore, mobile phase conditions may lead to poor peak separation and shifts in retention time, complicating compound identification. Finally, colored organic matter naturally present in the water may interfere with detection by UV-VIS (Beck *et al.*, 2018). Hence, other methods have been developed to enhance the sensitivity and specificity of the analysis. For instance, Xu et al. (2014), developed and validated an highly selective method for screening and confirmation organic explosive compounds with high performance liquid chromatography coupled with LTQ ion trap/Orbital mass spectrometric detections (HPLC-(PDA)-LTQOrbitrap). Similarly, Rapp-Wright et al. (2017) use liquid chromatography-high resolution accurate mass spectrometry (LC-HRMS) to screen and quantify the same compounds.

Improvements were also made on the detection of chemical warfare agents and their degradations products with the development of the HS-SPME-GC-MS/MS (Nawała *et al.*, 2016). These methods set the detection limits in the range of ng/L to µg/L. Limitations in some of the used techniques may thus suggest that the lack of detection of some munition constituents may represent a methodological artifact or lack of sensitivity rather than their absence in the environment. Regardless, several field studies have reported the detection of both parent compounds and metabolic products in dumpsites all over the world.

*Explosives and related chemicals*

Conventional explosives and corresponding metabolites have been measured in water and sediment samples in diverse geographical areas with reported concentrations varying from site to site (see Table 1). Conventional explosives thus far measured above detection limit values in water and/or sediment samples from several dumpsites are summarized in Table 1.

**Table 1. Physicochemical properties of conventional explosives and related chemicals detected in water and sediment samples from dumpsites.**

| Common chemical name or abbreviation | Class | Chemical Abstract Service Number | Molecular Weight (g/mol)[a] | Octanol-water partition coefficient (log Kow)[a] | Organic carbon-water partition coefficient - Koc (L/kg)[b] |
|---|---|---|---|---|---|
| 1,3-DNB | TNT metabolite | 99-65-0 | 168.12 | 1.55 | 351.6 |
| 1,3,5-TNB | Parent compound and TNT metabolite | 99-35-4 | 213.1 | 1.18 | 1683 |

| | | | | | |
|---|---|---|---|---|---|
| 2,4-DNT | Parent compound | 121-14-2 | 182.15 | 1.98 | 575.6 |
| 2,6-DNT | Parent compound | 606-20-2 | 182.5 | 2.02 | 587.4 |
| 2-ADNT | TNT metabolite | 35572-78-2 | 197.17 | 1.94 | 283 |
| 2-NT | TNT metabolite | 88-72-2 | 137.14 | 2.3 | 370.6 |
| 4-ADNT | TNT metabolite | 19406-51-0 | 197.17 | 1.91 | 283 |
| 4-NT | TNT metabolite | 99-99-0 | 137.14 | 2.3 | 363.2 |
| HMX | Parent compound | 2691-41-0 | 296.16 | 0.17 | 531.6 |
| NG | Parent compound | 55-63-0 | 227.11 | 1.62 | 115.8 |
| PETN | Parent compound | 78-11-5 | 316.17 | 3.17 | 647.9 |
| Picric acid | Tetryl metabolite | 88-89-1 | 229.10 | 1.33 | 2251 |
| RDX | Parent compound | 121-82-4 | 222.26 | 0.90 | 89.07 |
| Tetryl | Parent compound | 479-45-8 | 287.17 | 1.69 | 4605 |
| TNT | Parent compound | 118-96-7 | 227.13 | 1.6 | 2812 |

[a]Data retrieved from US EPA CompTox Chemical Dashboard (https://comptox.epa.gov/dashboard/), PubChem (https://pubchem.ncbi.nlm.nih.gov/).

[b]Data retrieved from KOCWIN, from EPI Suite v.4.1, developed by the United States Environmental Agency (USEPA).

In Europe, the presence of conventional explosives, both in water and sediment samples, has been reported in lake Mjøsa as well as coastal fortifications in Norway (Rossland *et al.*, 2010) and in the Swiss lakes of Thun and Brienz, as well as in important tributaries of such lakes

(Ochsenbein, Zeh and Berset, 2008). Likewise, in North America such compounds were found at sites along the west coast of the United States (USACE, 2013), the eastern coast of Canada (Rodacy *et al.*, 2001), Hawaiian coastal areas (UH, 2014b, 2014a; Briggs *et al.*, 2016) and in Puerto Rico (Porter, Barton and Torres, 2011). Detected concentrations vary from low µg/L up to dozens of mg/L, as reported by Porter et al. (Porter, Barton and Torres, 2011) for TNT and 2,4-DNT and 2,6-DNT in waters off Viesques Island, Puerto Rico (maximum detected concentration of approximately 86 and 83 mg/L, respectively). Concentrations in sediment samples have also been shown to vary from low µg/kg to hundreds of mg/kg, as exemplified by the 506 mg/kg of TNT measured at Viesques Island, Puerto Rico (Porter, Barton and Torres, 2011). Furthermore, large variation within sites may be observed in detected concentrations as a function of distance from the dumped munition. Such variability is thought to be caused by the filamentous nature of the plumes emanating from the source of contamination (Rodacy *et al.*, 2001; Camilli *et al.*, 2009). Additionally, a recent study showed that in situ measurements of dissolution fluxes from exposed munition material in the Baltic Sea are actually lower than the majority of those reported for laboratory experiments but clearly demonstrate that there is in fact chemical release from the dumped munition to the water column albeit lower than expected (Beck *et al.*, 2019). To better understand the occurrence of conventional explosives and related chemicals in the environment, Table 2 summarizes the range of concentrations thus far detected both in water and sediment samples as well as the percentage of environmental samples per sampling campaign in which such chemicals were detected within the collected samples, here expressed as detection frequency.

**Table 2. Overview of detected conventional explosives and related chemicals, minimum and maximum detected concentrations and associated detection frequencies from field water and sediment samples.**

| Chemical name or abbreviation | Range of concentrations measured in water samples (µg/L) | Range of detection frequency (%) | Range of concentrations measured in sediment samples (µg/kg) | Range of detection frequency (%) | Analytical method | References |
|---|---|---|---|---|---|---|
| 1,3-DNB | 13.6 - 18500 | NM | 3470 | NM | HPLC | (Barton and Porter, 2004; Porter, Barton and Torres, 2011) |
| | 1.02 – 5.9 | 7 | 90 | 22 | GC/ECD | (Rodacy *et al.*, 2001) |
| | 0.18 | NM | ND | - | HPLC and MS | (Rossland *et al.*, 2010) |
| 1,3,5-TNB | 8.15 - 11255 | NM | 30700 | NM | HPLC | (Barton and Porter, 2004; Porter, Barton and Torres, 2011) |
| | 0.10 – 1.24 | 9 | 90 | 96 | GC/ECD | (Rodacy *et al.*, 2001) |
| | 0.02 – 0.04 | 7 | ND | - | SPE | (Rosen *et al.*, 2017) |
| | ND | - | 220 – 250 | 5 | HPLC | (UH, 2014a) |
| 2,4-DNT | 58 - 82500* | NM | 26000* | NM | HPLC | (Barton and Porter, 2004; Porter, Barton and Torres, 2011) |
| | 0.02 – 3.14 | 50 | 560 | 48 | GC/ECD | (Rodacy *et al.*, 2001) |
| | 0.01 – 0.07 | 7 | ND | - | SPE | (Rosen *et al.*, 2017) |
| | ND | - | 30 – 3300 | 61 | HPLC | (UH, 2014a) |
| | ND | - | 30 - 110000 | 55 | HPLC | (UH, 2014b) |
| | ND | - | 200 – 970 | 25 | HPLC and LC-MS/MS | (USACE, 2013) |
| 2,6-DNT | 58 - 82500* | NM | 26000* | NM | HPLC | (Barton and Porter, 2004; Porter, Barton and Torres, 2011) |
| | 1.7 - 2.0 | 8 | 850 | 35 | GC/ECD | (Rodacy *et al.*, 2001) |
| | ND | - | 98 – 380 | 11 | HPLC | (UH, 2014a) |

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|---|---|---|---|---|---|---|
| | ND | - | 70 - 10000 | 21 | HPLC | (UH, 2014b) |
| | ND | - | 120 | 8 | HPLC and LC-MS/MS | (USACE, 2013) |
| 2-ADNT | 0.04 - 108 | NM | 90 | 30 - 38 | GC/ECD | (Rodacy *et al.*, 2001) |
| | 0.004 – 0.26 | 14 | ND | - | SPE | (Rosen *et al.*, 2017) |
| | 0.1 | NM | ND | - | HPLC and MS | (Rossland *et al.*, 2010) |
| 2-NT | 26.4 - 40500 | NM | ND | - | HPLC | (Barton and Porter, 2004; Porter, Barton and Torres, 2011) |
| | ND | - | 80 – 1100 | 4 | HPLC | (UH, 2014b) |
| 4-ADNT | 0.03 – 123 | NM | 550 | 39 - 46 | GC/ECD | (Rodacy *et al.*, 2001) |
| | 0.02 – 0.32 | 7 | ND | - | SPE | (Rosen *et al.*, 2017) |
| | 0.32 | NM | ND | - | HPLC and MS | (Rossland *et al.*, 2010) |
| 4-NT | ND | - | 5390 | NM | HPLC | (Barton and Porter, 2004; Porter, Barton and Torres, 2011) |
| | ND | - | 90 – 120 | NM | HPLC | (Briggs *et al.*, 2016) |
| | ND | - | 490 | 2 | HPLC | (UH, 2014b) |
| HMX | 0.0001 – 0.62 | NM | 60 – 290 | NM | HPLC | (Ampleman *et al.*, 2004) |
| | 0.0004 – 0.0009 | NM | ND | - | LC-MS/MS | (Ochsenbein, Zeh and Berset, 2008) |
| | 0.62 | NM | 60 – 410 | NM | HPLC and MS | (Rossland *et al.*, 2010) |
| NG | ND | - | 560 – 1700 | 2 | HPLC | (UH, 2014b) |
| PETN | 0.0004 – 0.0009 | NM | ND | - | LC-MS-MS | (Ochsenbein, Zeh and Berset, 2008) |
| Picric acid | ND | - | 3 | 8 | HPLC and LC-MS/MS | (USACE, 2013) |
| RDX | 3.28 - 4120 | NM | 5320 | NM | HPLC | (Barton and Porter, 2004; |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | Porter, Barton and Torres, 2011) |
| | 0.0004 – 0.0009 | NM | ND | - | LC-MS-MS | (Ochsenbein, Zeh and Berset, 2008) |
| | 0.004 – 0.05 | 10 - 79 | ND | - | SPE | (Rosen *et al.*, 2017) |
| | 12.7 | NM | 50 | NM | HPLC and MS | (Rossland *et al.*, 2010) |
| | ND | - | 140 | 2 | HPLC | (UH, 2014b) |
| | ND | - | 580 – 590 | 17 | HPLC and LC-MS/MS | (USACE, 2013) |
| Tetryl | ND | - | 230 | 8 | HPLC and LC-MS/MS | (USACE, 2013) |
| TNT | 7.87 - 85700 | NM | 19333000 | NM | HPLC | (Barton and Porter, 2004; Porter, Barton and Torres, 2011) |
| | 0.01 – 14.2 | 25 | 170 | 100 | GC/ECD | (Rodacy *et al.*, 2001) |
| | 0.006 – 7.5 | 7 | ND | - | SPE | (Rosen *et al.*, 2017) |
| | 0.17 – 0.2 | NM | 50 – 310 | NM | HPLC and MS | (Rossland *et al.*, 2010) |

ND – not detected.

NM – not mentioned.

* – The concentrations of 2,4-DNT and 2,6-DNT are reported together in Barton and Porter (2004) and Porter, Barton and Torres (2011).

*Chemical warfare agents and related chemicals*

Also CWAs have been detected in environmental samples, both in the water column and in the sediment. CWA and related chemicals detected at selected marine sites are presented in Table 3.

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280 **Table 3. Physicochemical properties of chemicals warfare agents and related chemicals**

281 **thus far detected in water and sediment samples from dumpsites in the Baltic Sea and the**

282 **dumpsite off the Hawaiian coast HI-05.**

| Common chemical name or abbreviation | Class | Chemical Abstract Service Number | Molecular Weight (g/mol)[a] | Octanol-water partition coefficient (log Kow)[a] | Organic carbon-water partition coefficient - Koc (L/kg)[b] |
|---|---|---|---|---|---|
| α-chloroacetophenone | Parent compound | 532-27-4 | 154.59 | 2.02 | 98.9 |
| 1,4-dithiane | Sulfur mustard metabolite | 505-29-3 | 120.23 | 0.77 | 145.8 |
| 1,4-oxithiane | Sulfur mustard metabolite | 15980-15-1 | 104.17 | 0.742 | 19.59 |
| 1,4,5-oxadithiepane | Sulfur mustard metabolite | 3886-40-6 | 136.23 | 1.40 | NA |
| 1,2,5-trithiepane | Sulfur mustard metabolite | 6576-93-8 | 152.29 | 2.19 | NA |
| 2-Chlorovinylarsonic acid | Lewisite metabolite | 64038-44-4 | 186.42 | -0.472 | 14.65 |
| Bis(2-chlorovinyl)arsinic acid | Lewisite metabolite | 157184-21-9 | 230.91 | 1.79 | NA |
| Bis(diphenylarsinyl) oxide | Clark I metabolite | 2215-16-9 | 474.266 | 6.93 | NA |
| Clark I | Parent compound | 712-48-1 | 264.58 | 4.52 | 15300 |
| Diphenylarsinic acid | Clark I metabolite | 4656-80-8 | 262.14 | 2.80 | NA |
| Phenylarsonic acid | PDCA metabolite | 98-05-5 | 202.04 | 0.03 | 1001 |
| Phenarsazinic acid | Adamsite metabolite | 4733-19-1 | 275.13 | 2.33 | NA |
| Sulphur mustard | Parent compound | 505-60-2 | 159.07 | 2.28 | 239.7 |
| Triphenylarsine | Parent compound | 603-32-7 | 306.24 | 5.97 | 335600 |

| | | | | | |
|---|---|---|---|---|---|
| Triphenylarsine oxide | Triphenylarsine metabolite | 1153-05-5 | 322.239 | 5.97 | 520700 |
| Thiodiglycol sulfoxide | Sulfur mustard metabolite | 3085-45-8 | 138.18 | -2.23 | 1 |
| Thiodiglycolic acid | Sulfur mustard metabolite | 123-93-3 | 150.16 | -0.435 | 13.36 |

[a]Data retrieved from US EPA CompTox Chemical Dashboard (https://comptox.epa.gov/dashboard/), PubChem (https://pubchem.ncbi.nlm.nih.gov/) and Christensen et al. (2016).

[b]Data retrieved from KOCWIN, from EPI Suite v.4.1, developed by the United States Environmental Agency (USEPA).

NA – not available.

Unlike the conventional explosives, for which studies on their occurrence in the environment cover various areas of the globe, the presence of CWAs has been of particular interest in European dumpsites, with extensive studies in the Baltic Sea through projects as Modeling of Ecological Risks Related to Sea-Dumped Chemical Weapons (MERCW) (https://cg.cs.uni-bonn.de/en/projects/mercw/) (Missiaen *et al.*, 2010; Sanderson *et al.*, 2010), Nord Stream (Sanderson *et al.*, 2014) and Chemical Munitions Search & Assessment (CHEMSEA) (Bełdowski, Klusek, *et al.*, 2016), in the North Sea (a dumpsite in Skagerrak) (Tørnes *et al.*, 2002; Tørnes, Opstad and Johnsen, 2006) and in the Adriatic Sea (Amato *et al.*, 2006). Also a dumpsite off the Hawaiian coast (HI-05) was studied in the context of the Hawaii Undersea Military Munitions Assessment (HUMMA) project (Briggs *et al.*, 2016). Interestingly, all these studies show that parent CWA compounds are rarely detected. Conversely their metabolites are found in up to 81% of the collected sediment samples (Sanderson *et al.*, 2012, 2014). CWA concentrations in the sediment varied between low µg/kg to a maximum of 81 mg/kg of

triphenylarsine, measured within the MERCW project (Missiaen *et al.*, 2010; Sanderson *et al.*, 2010). Moreover, the analysis of sediment samples collected in 2009 and 2012 at the HI-05 dumpsite, both part of HUMMA project, not only confirm the contamination of the sediment by CWAs but also reveal a sharp increase in the reported concentrations. This not only suggests that dumped munition may in fact be leaking toxic chemicals to the surrounding environment but also that these chemicals may be capable of binding to sediment particles and persist for years (Briggs *et al.*, 2016). Furthermore, non-targeted screening of marine sediment samples carried out by Niemikoski and colleagues (2020) resulted in the detection and identification of 14 previously unknown CWA-related phenylarsenic chemicals. While exact concentrations could not be determined within the framework of the study, it still provides essential information for marine risk assessment and highlights the need for more sensitive analytical tools (Niemikoski, Sö, *et al.*, 2020). In the water samples collected in the Baltic Sea, the lowest measured concentration was 4 µg/L of 1,2,5-trithiepane while the highest was the 1.5 mg/L of diphenylarsinic acid (Missiaen *et al.*, 2010; Sanderson *et al.*, 2010, 2014; Bełdowski, Klusek, *et al.*, 2016). Vanninen et al. 2020 have recently estimated that CWAs pollution may spread out more than 250 meters from the dumped munitions source (Vanninen *et al.*, 2020). An overview of detected chemical warfare agents and related chemicals in the environment, including measured concentrations in water and sediment samples and range of detection frequencies, is provided in Table 4.

**Table 4. Overview of detected chemical warfare agents and metabolites, minimum and maximum detected concentrations and associated detection frequencies from field water and sediment samples.**

| Chemical name or abbreviation | Range of concentrations measured in water samples (µg/L) | Range of detection frequency (%) | Range of concentrations measured in sediment samples (µg/kg) | Range of detection frequency (%) | Analytical method | References |
|---|---|---|---|---|---|---|
| α-chloroacetophenone | ND | - | 7.5 | 1.1 | GC-MS, LC-MS/MS and GC-MS/MS | (Bełdowski, Szubska, *et al.*, 2016) |
| 1,4-dithiane | ND | - | 18 - 2100 | 5.4 – 33.2 | GC-MS, LC-MS/MS, GC-MS/MS and QMS | (Tørnes *et al.*, 2002; Amato *et al.*, 2006; Bełdowski, Szubska, *et al.*, 2016; Briggs *et al.*, 2016) |
| 1,4-oxithiane | ND | - | 87 - 1100 | 0.7 - 1.6 | GC-MS, LC-MS/MS, GC-MS/MS and QMS | (Tørnes *et al.*, 2002; Amato *et al.*, 2006; Bełdowski, Szubska, *et al.*, 2016; Briggs *et al.*, 2016) |
| 1,4,5-oxadithiepane | 19 | 1.1 | 40 - 700 | 8.7 | GC-MS, LC-MS/MS, GC-MS/MS and QMS | (Tørnes *et al.*, 2002; Bełdowski, Szubska, *et al.*, 2016) |
| 1,2,5-trithiepane | 3.4 | 0.5 | 35 - 600 | 8.7 | GC-MS, LC-MS/MS, GC-MS/MS and QMS | (Tørnes *et al.*, 2002; Bełdowski, Szubska, *et al.*, 2016) |
| 2-Chlorovinylarsonic acid | ND | - | 54.9 | 2 | GC-MS and LS-MS/MS | (Sanderson *et al.*, 2014) |
| Bis (2-chlorovinyl)arsinic acid | ND | - | 70.3 | 2 | GC-MS and LS-MS/MS | (Sanderson *et al.*, 2014) |
| Bis(diphenylarsinyl) oxide | ND | - | 200 - 137000 | | QMS | (Tørnes *et al.*, 2002) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Clark I | ND | - | 100 - 178000 | | QMS | (Tørnes *et al.*, 2002) |
| Diphenylarsinic acid | 940 – 1538 | 2.2 – 5.2 | 140 – 9583 | 8.2 - 50 | GC-MS, LC-MS/MS and GC-MS/MS | (Missiaen *et al.*, 2010; Sanderson *et al.*, 2010, 2014; Bełdowski, Szubska, *et al.*, 2016) |
| Phenylarsonic acid | 4 – 442 | 2.2 – 5.2 | 327 – 10833 | 2 - 81 | GC-MS, LC-MS/MS and GC-MS/MS | (Missiaen *et al.*, 2010; Sanderson *et al.*, 2010, 2014; Bełdowski, Szubska, *et al.*, 2016) |
| Phenarsazinic acid | 17 | 1.1 | 200 – 1400 | 3.5 – 62.1 | GC-MS, LC-MS/MS and GC-MS/MS | (Missiaen *et al.*, 2010; Sanderson *et al.*, 2010, 2014; Bełdowski, Szubska, *et al.*, 2016) |
| Sulphur mustard | ND | - | 2.1 – 2400 | 1.1 – 19.9 | GC-MS and QMS | (Tørnes *et al.*, 2002; Briggs *et al.*, 2016) |
| Triphenylarsine | 68 | 2.7 | 20 - 81250 | 8.7 – 32.8 | GC-MS, LC-MS/MS, GC-MS/MS and QMS | (Tørnes *et al.*, 2002; Missiaen *et al.*, 2010; Sanderson *et al.*, 2010; Bełdowski, Szubska, *et al.*, 2016) |
| Triphenylarsine oxide | 20 | 1.35 | 590 | 4.3 | GC-MS, LC-MS/MS and GC-MS/MS | (Bełdowski, Szubska, *et al.*, 2016) |
| Thiodiglycol sulfoxide | ND | - | 3.3 – 610 | 1.7 – 2.1 | GC-MS, LC-MS/MS | (Missiaen *et al.*, 2010; |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | and GC-MS/MS | Sanderson *et al.*, 2010; Bełdowski, Szubska, *et al.*, 2016) |
| Thiodiglycolic acid | ND | - | 550 | 1.1 | GC-MS, LC-MS/MS and GC-MS/MS | (Bełdowski, Szubska, *et al.*, 2016) |

ND – not detected.

NM – not mentioned.

## 4. Adverse effects of munition constituents to environmental and human health

The presence of both conventional and chemical munition constituents in the aquatic environment may cause adverse effects on resident marine organisms and ecosystems. Toxicity data for conventional and chemical munition constituents previously detected in environmental samples are presented in Table 5 and Table 6. Currently available toxicity data for humans, fish, copepods, algae and bacteria are compiled, providing an overview on different sensitivities to the various compounds across a wide range of organisms occupying different trophic levels. Given the high number of toxicity data entries available for some of the conventional explosives and corresponding metabolites, a selection of toxicity values per trophic level, covering relevant endpoints and species is provided, hence providing an overview of the toxic potential of such chemicals while avoiding an over-extensive and redundant presentation of data. Data on aquatic organisms represent both marine and freshwater species, with special focus on the first as most of the dumpsites are located in the marine environment. Human toxicity data was retrieved from ToxCast (Dix *et al.*, 2007) and Tox21 (Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013), compiled under the CompTox Chemistry Dashboard (Williams *et al.*, 2017), both programs providing high-throughput screening *in vitro* toxicity data targeting lower, yet more

346 sensitive, levels of biological organization. This approach hence allows the identification of the
347 molecular initiating events triggered by the stressors which may then be linked to the to the
348 final adverse outcome to the organisms, yet at much lower concentrations and earlier stages,
349 via the in-constant-development Adverse Outcome Pathways (AOPs).

350

351 **Table 5. Summary of the currently available data on the effects of conventional explosives**
352 **and corresponding metabolites, with particular focus on humans, fish, copepods, algae**
353 **and bacteria.**

| Chemical | Taxonomic group | Species | Reported effects | References |
|---|---|---|---|---|
| 1,3,5-TNB | Fish | *Oncorhynchus mykiss* | LOEC of 0.17 mg/L for both growth and survival after 71 days exposure. | (van der Schalie, 1983) |
| | | *Cyprinodon variegatus* | LC50 of 1.20 mg/L after 5 days exposure. | (Lotufo, Blackburn and Gibson, 2010) |
| | Algae | *Ulva fasciata* | LOEC of 0.053 mg/L for zoospore germination after 4 days exposure. | (Nipper *et al.*, 2001) |
| | | *Pseudokirchneriella subcapitata* | LOEC of 1.18 mg/L for population growth after 5 days exposure. | (van der Schalie, 1983) |
| 1,3-DNB | Human | *Homo sapiens* | AC50 of 25.2 µM for anticoagulant rodenticide inhibition of vitamin K epoxide reductase resulting in coagulopathy and hemorrhage (Event 1134, AOP 187). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | | | AC50 of 35.8 µM for estrogen receptor activation leading to breast cancer (Event 1181, AOP 200). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | Fish | *Oncorhynchus mykiss* | LC50 of 1.7 mg/L after 4 days exposure. | (van der Schalie, 1983) |
| | | *Sciaenops ocellatus* | LOEC of 49.6 mg/L for larvae survival after 2 days exposure. | (Nipper *et al.*, 2001) |

| | | | | |
|---|---|---|---|---|
| | Algae | *Ulva fasciata* | LOEC of 0.62 mg/L for zoospore germination after 4 days exposure. | (Nipper *et al.*, 2001) |
| | | *Pseudokirchneriella subcapitata* | LOEC of 0.97 mg/L for population growth after 5 days exposure. | (van der Schalie, 1983) |
| TNT | Fish | *Oncorhynchus mykiss* | LOEC of 0.49 mg/L for survival after 60 days exposure. | (Bailey *et al.*, 1985) |
| | | *Danio rerio* | LC50 of 4.5 mg/L after 5 days exposure. | (Koske, Goldenstein and Kammann, 2019) |
| | | *Platichtys flesus* | Half maximum inhibitory concentration (IC50) for EROD activity of 28.1 µM and 37.7 µM for MROD activity. | (Koske, Goldenstein, *et al.*, 2020) |
| | Copepods | *Nitocra spinipes* | LC50 of 7.6 mg/L after 4 days exposure. | (Dave, Nilsson and Wernersson, 2000) |
| | | *Tigriopus japonicus* | LC50 of 4.8 mg/L after 4 days exposure. | (Liang *et al.*, 2017) |
| | Algae | *Ulva fasciata* | LOEC of 2.9 mg/L for zoospore germination after 4 days exposure. | (Nipper *et al.*, 2001) |
| | | *Pseudokirchneriella subcapitata* | LOEC of 4.1 mg/L for population growth after 14 days exposure. | (Liu *et al.*, 1983b) |
| | Bacteria | *Anabaena flosaquae* | LOEC of 8.2 mg/L for population growth after 14 days exposure. | (Liu *et al.*, 1983b) |
| | | *Microcystis (Diplocystis) aeruginosa* | LOEC of 4.1 mg/L for population growth after 14 days exposure. | (Liu *et al.*, 1983b) |
| Picric acid | Fish | *Oncorhynchus mykiss* | LC50 of 110 mg/L after 4 days exposure. | (Goodfellow *et al.*, 1983) |
| | | *Cyprinodon variegatus* | LC50 of 130 mg/L after 4 days exposure. | (Heitmuller, Hollister and Parrish, 1981) |
| | Copepod | *Nitocra spinipes* | LC50 of 92 mg/L after 4 days exposure. | (Dave, Nilsson and Wernersson, 2000) |
| | Algae | *Ulva fasciata* | LOEC of 336 mg/L for zoospore germination after 4 days exposure. | (Nipper *et al.*, 2001) |

| | | | | |
|---|---|---|---|---|
| Tetryl | Fish | *Sciaenops ocellatus* | LOEC of 1.1 mg/L for larvae survival after 2 days exposure. | (Nipper *et al.*, 2001) |
| | Algae | *Ulva fasciata* | LOEC of 0.67 mg/L for zoospore germination after 4 days exposure. | (Nipper *et al.*, 2001) |
| 2,4-DNT | Human | *Homo sapiens* | AC50 of 26.4 µM for anticoagulant rodenticide inhibition of vitamin K epoxide reductase resulting in coagulopathy and hemorrhage (Event 1134, AOP 187). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | | | AC50 of 28.3 µM for acetylcholinesterase inhibition leading to acute mortality (Event 12, AOP 16). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | Fish | *Oncorhynchus mykiss* | LC50 of 16.3 mg/L after 4 days exposure. | (Liu *et al.*, 1983a) |
| | | *Gasterosteus aculeatus* | LC50 of 2.2 mg/L after 35 days exposure. | (van den Dikkenberg *et al.*, 1989) |
| | Copepod | *Nitocra spinipes* | LC50 of 17.0 mg/L after 4 days exposure. | (Dave, Nilsson and Wernersson, 2000) |
| | Algae | *Ulva fasciata* | LOEC of 4.40 mg/L for zoospore germination after 4 days exposure. | (Nipper *et al.*, 2001) |
| | Bacteria | *Microcystis (Diplocystis) aeruginosa* | LOEC of 0.50 mg/L for population growth after 14 days exposure. | (Liu *et al.*, 1983b) |
| 2,6-DNT | Human | *Homo sapiens* | AC50 of 29.4 µM for acetylcholinesterase inhibition leading to acute mortality (Event 12, AOP 16). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | | | AC50 of 29.4 µM for NR1I2 activation leading to hepatic steatosis (Event 245, AOP 60). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | Fish | *Sciaenops ocellatus* | LOEC of 31 mg/L for larvae survival after 2 days exposure. | (Nipper *et al.*, 2001) |
| | Copepod | *Schizopera knabeni* | LC50 of 65 mg/L after 4 days exposure. | (Nipper *et al.*, 2005) |

| | | | | |
|---|---|---|---|---|
| | Algae | *Ulva fasciata* | LOEC of 4.40 mg/L for zoospore germination after 4 days exposure. | (Nipper *et al.*, 2001) |
| 2-ADNT | Fish | *Cyprinodon variegatus* | LC50 of 8.6 mg/L after 5 days exposure. | (Lotufo, Blackburn and Gibson, 2010) |
| | | *Danio rerio* | LC50 of 13.4 mg/L after 4 days exposure. | (Koske, Goldenstein and Kammann, 2019) |
| 2-NT | Human | *Homo sapiens* | AC50 of 0.652 µM for anticoagulant rodenticide inhibition of vitamin K epoxide reductase resulting in coagulopathy and hemorrhage (Event 1134, AOP 187). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | | | AC50 of 31.9 µM for aromatase inhibition leading to ovulation inhibition and decreased fertility (Event 964, AOP 153). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | Fish | *Pimephales promelas* | LC50 of 38 mg/L after 4 days exposure. | (Pearson *et al.*, 1979) |
| | | *Danio rerio* | EC50 of 28 mg/L for reproduction after 7 days exposure. | (Maas-Diepeveen and van Leeuwen, 1986) |
| | Algae | *Chlorella pyrenoidosa* | LOEC of 8.7 mg/L for population growth after 3 days exposure. | (Ramos *et al.*, 1999) |
| | | *Chlorella pyrenoidosa* | EC50 of 22 mg/L for population growth after 3 days exposure. | (Ramos *et al.*, 1999) |
| 4-ADNT | Fish | *Pimephales promelas* | LC50 of 6.9 mg/L after 4 days exposure. | (Pearson *et al.*, 1979) |
| | | *Danio rerio* | LC50 of 14.4 mg/L after 4 days exposure. | (Koske, Goldenstein and Kammann, 2019) |
| 4-NT | Human | *Homo sapiens* | AC50 of 47.4 µM for NR1I2 activation leading to hepatic steatosis (Event 245, AOP 60). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | | | AC50 of 4.13 µM for altered ion channel activity leading to impaired heart function (Event 697, AOP 104). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |

| | | | | |
|---|---|---|---|---|
| | Fish | *Poecilia reticulata* | LC50 of 36.9 mg/L after 14 days exposure. | (Maas-Diepeveen and van Leeuwen, 1986) |
| | | *Pimephales promelas* | LC50 of 49.9 mg/L after 4 days exposure. | (Pearson *et al.*, 1979) |
| | Algae | *Scenedesmus quadricauda* | LOEC of 15 mg/L for population growth after 7 days exposure. | (Bringmann and Kühn, 1980) |
| RDX | Fish | *Cyprinodon variegatus* | LC50 of 9.9 mg/L after 5 days exposure. | (Lotufo, Blackburn and Gibson, 2010) |
| | | *Pimephales promelas* | LOEC of 3.5 mg/L for survival after 4 days exposure. | (Warner *et al.*, 2012) |
| | | *Pimephales promelas* | LOEC of 1.75 mg/L for vertebral deformity after 4 days exposure. | (Warner *et al.*, 2012) |
| | Algae | *Ulva fasciata* | LOEC of 15.7 mg/L for zoospore germination after 4 days exposure. | (Nipper *et al.*, 2001) |
| NG | Fish | *Pimephales promelas* | LOEC of 0.200 mg/L for hatching success after 28 days exposure. | (Burton, Turley and Peters, 1993) |
| | | *Oncorhynchus mykiss* | LC50 of 1.9 mg/L after 4 days exposure. | (Burton, Turley and Peters, 1993) |
| | | *Oncorhynchus mykiss* | LOEC of 0.06 mg/L for growth after 60 days exposure. | (Burton, Turley and Peters, 1993) |
| HMX | Fish | *Pimephales promelas* | LC50 of 15 mg/L after 4 days exposure. | (Bentley *et al.*, 1977) |
| PETN | Human | *Homo sapiens* | AC50 of 15.0 µM for anticoagulant rodenticide inhibition of vitamin K epoxide reductase resulting in coagulopathy and hemorrhage (Event 1134, AOP 187). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | | | AC50 of 2.08 µM for estrogen receptor activation leading to breast cancer (Event 1181, AOP 200). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |

| | | | |
|---|---|---|---|
| | Fish | *Pimephales promelas* | LC50 of 2.7% v/v after 4 days exposure. (Bentley, Sleight and Macek, 1975) |

In general, the gathered data is evenly distributed across the selected representative taxonomic groups; for most the compounds toxicity data are available for at least three taxonomic groups. For 2-ADNT (CASRN 35572-78-2), 4-ADNT (CASRN 19406-51-0), NG (CASRN 55-63-0) and HMX (CASRN 2691-41-0) only data for fish species are available, revealing a gap in the current knowledge that must be overcome in order to better understand the toxic potential of these compounds across species and thus to allow better assessment of their environmental impact. Moreover, the information present in Table 5 shows that all compounds exert lethal effects to half of the exposed organisms, represented as LC50, at the low mg/L mostly after 4 days of exposure, except for picric acid for which LC50 values are in the range of a hundred mg/L. Not surprisingly, both the Lowest Observed Effect Concentration (LOEC) and concentration affecting 50% of the test organisms (EC50) values vary depending on the endpoint and the exposure period. These concentrations rarely exceed a few mg/L. Furthermore, both lethal and non-lethal toxicity values are in line with the concentrations detected in various environmental samples collected immediately above the dumped munition (distance = 0 meters), indicating that organisms inhabiting areas impacted by dumpsites are directly exposes to such chemicals hence potentially being severely affected (see Table 2 and Table 5). Nonetheless, as the distance from the munition increases the dispersion and dilution of the compounds leads to a decrease in the detected concentrations to values in the range of µg/L (see Table 2), hence sharply reducing the risk posed by such compounds.

Two reports by Lotufo and colleagues extensively discuss the occurrence and toxicity of conventional explosives and related chemicals with particular focus on dumpsites located in

North America (Lotufo *et al.*, 2017; Lotufo, Rosen and Carton, 2021). Further, following USEPA methods for deriving ambient water quality criteria (WQC) for the protection of aquatic life (https://www.epa.gov/wqc/basic-information-water-quality-criteria), Lotufo et al. (2017) derived both acute and chronic WQC for several conventional explosives (which can be considered ecotoxicological thresholds for environmental concentrations)(Lotufo *et al.*, 2017). In addition, values derived in previous studies are also summarized in this highly-cited report allowing the compilation of WQC values for TNT, 2-ADNT, 4-ADNT, 1,3,5-TNB, 1,3-DNB, 3,5-DNA, HMX, RDX, NG, and NC. The derived WQC values vary greatly depending on the compound, acute or chronic exposure period and endpoint and type of (marine or freshwater) environment. Values range from low µg/L to dozens of mg/L, being the thresholds for chronic or acute exposure, respectively. For instance, freshwater WQC for TNT are, according to Lotufo et al. (2017), 230 and 73 µg/L, for acute and chronic exposure, while Talmage et al. (1999) report those for RDX to be of 1,390 and 186 µg/L, respectively. Moreover, even though the availability of marine WQC values is rather limited, data suggests an enhanced sensitivity of marine organisms to munition constituents, when compared to freshwater organisms, suggesting these substances may impact marine organisms at even lower concentrations (Talmage *et al.*, 1999; Lotufo *et al.*, 2017). Despite the reported WQC values being either within or close to the concentrations detected in the environment for many of the compounds, Lotufo et al. (2017) still conclude that the currently available toxicity data and measured environmental concentrations suggest that the risks to aquatic invertebrates and fish are negligible mainly due to rapid dilution of the compounds in the water column and the general low sensitivity of the organisms to the tested endpoints. In fact, as part of the CHEMSEA project, Kotwicki et al. (2016) studied that nematode community structures from Bornholm Deep, Gotland Deep and Gdansk Deep, in the Baltic Sea, and observed significant difference in the structure of the communities inhabiting the dumpsites compared to the reference site (Kotwicki, Grzelak and

Bełdowski, 2016). Similarly, studies by Ek and colleagues suggest that, when the ammunition is not buried in sediment, there is a rapid release of TNT up to acutely toxic concentrations (Ek *et al.*, 2007; Ek, Nilsson and Dave, 2008). Nevertheless, given the limited understanding regarding the munition-related toxicity, chemical release to the sediment and water column rate and chemical dilution rate, precautionary conclusions should be taken on the risks posed by conventional explosives to aquatic organisms. Further, human in vitro toxicity data collected from ToxCast/Tox21 indicates that several molecular events are impacted at concentrations as low as ng/L, and others at the low µg/L. These events are linked, via AOPs, to adverse outcomes as severe as ovulation inhibition and decreased fertility, impaired heart rate, hemorrhage and breast cancer, clearly not only having an impact in the fitness of the individual but also potentially affecting the species at a population level. Interestingly, a case study on civilian exposure to munition-specific carcinogens revealed a significant increase in the lung and bronchus cancer incidence rates that were correlated with high munition exposure of a population from the Puerto Rican island of Visques. In this case, the lungs and bronchus were specifically affected because the primary exposure route was inhalation (Sanderson *et al.*, 2017). Similarly, the in vitro study carried out by Koske et al. on TNT interaction with CYP1A enzymes clearly show an interference in EROD and MROD activities following exposure to concentrations as low as 0.6 mg/L (Koske, Goldenstein, *et al.*, 2020). Even though the use of in vitro data for risk assessment purposes still lacks adequate guidelines, its application for the identification and comparison of chemicals' hazard properties is generally accepted (Fay *et al.*, 2017). For instance, ToxCast/Tox21 was already used to identify the hazard to human health posed by suspect chemicals detected in house dust (Rager *et al.*, 2016) as well as in different water matrices (Brunner *et al.*, 2019; Barbosa *et al.*, 2020). As such, the in vitro toxicity data presented here suggest that the use of traditional endpoints, as those used for the calculation of the previously referred WQC, may lead to an under-estimation of the toxic potential of

conventional explosives detected in the environment and simultaneously reinforce the importance of shifting to lower levels of biological complexity which allow the detection of adverse effects at lower concentrations. Indeed, lower levels of biological complexity can provide essential information on the mechanisms specifically impacted by each stressor, which can ultimately be linked to adverse outcomes at the organism level via AOPs. This will allow to model, predict and estimate effects in more realistic exposure scenarios where organisms are exposed to low concentrations of single or mixtures of chemicals for very prolonged periods of time.

More recently, the research focus has moved from the determination of toxicity values towards the study of the toxicokinetics of conventional explosives, specifically TNT and metabolites. Mariussen et al. (2018), for example, used labeled TNT (14C TNT) to study its uptake and excretion by juvenile Atlantic salmon (*Salmo salar*). Results show a rapid increase in the 14C TNT uptake in gills, blood, liver, kidney, muscle and brain, with a maximum tissue concentration after 6 h exposure. Subsequently, even though the fish were still exposed to 14C TNT in the water, a decrease was detected in the internal concentration as a result of the excretion from the fish. By the end of the experiment a reduction of 12% in the initial total radioactivity was detected, presumably due to uptake and sorption by the fish, but may also be related to adsorption to the exposure tank walls made of polyethylene which has hydrophobic properties. Because of the rapid excretion and estimated bioconcentration factors, the authors suggest a low potential for bioaccumulation of TNT. Nonetheless, the same study also reported hemorrhages in the dorsal muscle tissues near the spine as well as the impairment of physiological parameters in blood such as glucose, urea, HCO3, Cl and hemoglobin levels (Mariussen *et al.,* 2018). The bioaccumulation of TNT and its most commonly detected metabolites, 2-ADNT and 4-ADNT, was also studied in transplanted blue mussels (*Mytilus edulis*). In this study, six moorings with mussels bags were placed east and west of a mine

mound at a dumpsite in Kolberger Heide, Germany. After three independent exposure periods of 106, 146 and 92 days, only 4-ADNT was found in mussels' tissue ranging from 2.40 ± 2.13 to 7.76 ± 1.97 ng/g (wet weight). TNT and 2-ADNT were not detected (Appel *et al.*, 2018). In a very similar study, Strehse et al. 2017 monitored the presence of TNT, 2-ADNT and 4-ADNT in the marine environment with transplanted blue mussel yet this time with a single 93 day exposure period but positioning mussels both on the seafloor of a dumpsite impacted area and one meter above it. All three compounds were detected in the mussels placed on the seafloor at concentration as high as 131.31 ± 9.53 ng/g (wet weight). For those placed one meter above the seafloor, only 4-ADNT was detected (8.71 ± 2.88 ng/g mussel wet weight) (Strehse *et al.*, 2017). Also Koske et al. (2020) detected different conventional munition-related chemicals in the bile of dab (*Limanda limanda* L.) of fish collected in the vicinity of a dumpsite in the Baltic Sea (Koske, Straumer, *et al.*, 2020). More recently, Beck et al. (2022) collected marine biota, including macroalgae, molluscs, crustacean and fish, from the Kiel Bight and the dumpsite at Kolberger Heide aiming at studying the bioaccumulation potential of explosives and related chemicals. Such chemicals were detected across the collected organisms at concentration in the range of 1 ng/g hence providing evidence of the widespread bioaccumulation of explosives and related chemicals in the marine food web (Beck *et al.*, 2022). These studies show that, even though at low concentrations, these conventional explosives accumulate in the marine biota therefore posing a threat to the marine ecosystem and human health. Finally, the usefulness of marine bivalves as bioindicators of pollution at dumped munitions in the marine environment is summarized in the recent review by Strehse and Maser (Strehse and Maser, 2020).

**Table 6. Summary of the currently available data on the effects of chemicals munition constituents and related chemicals, with particular focus on humans, fish, copepods, algae and bacteria.**

| Chemical | Taxonomic group | Species | Reported effects | References |
|---|---|---|---|---|
| α-chloroacetophenone | Human | *Homo sapiens* | AC50 of 18.5 µM for NR1I3 suppression leading to hepatic steatosis (Event 167, AOP 58). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | | | AC50 of 24.1 µM for estrogen receptor activation leading to breast cancer (Event 1181, AOP 200). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | Bacteria | *Allivibrio fischeri* | EC50 of 0.0112 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |
| 1,4-dithiane | Human | *Homo sapiens* | AC50 of 68.4 µM for constitutive androstane receptor activation leading to hepatocellular adenomas and carcinomas (Event 715, AOP 107). | (Dix *et al.*, 2007; Attene-Ramos *et al.*, 2013; Tice *et al.*, 2013) |
| | Bacteria | *Allivibrio fischeri* | EC50 of 9.97 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |
| | Crustacean | *Daphnia magna* | NOEC of 28.7 mg/L after 2 days exposure. | (Czub, Nawała, Popiel, Dziedzic, *et al.*, 2020) |
| 1,4-oxithiane | Bacteria | *Allivibrio fischeri* | EC50 of 47.4 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |
| | Crustacean | *Daphnia magna* | NOEC of 108.8 mg/L after 2 days exposure. | (Czub, Nawała, Popiel, Dziedzic, *et al.*, 2020) |
| 1,4,5-oxadithiepane | Bacteria | *Allivibrio fischeri* | EC50 of 1.70 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |
| | Crustacean | *Daphnia magna* | LC50 of 2.188 mg/L after 2 days exposure. | (Czub, Nawała, Popiel, Dziedzic, *et al.*, 2020) |

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| 1,2,5-trithiepane | Bacteria | *Allivibrio fischeri* | EC50 of 1.17 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |
| | Crustacean | *Daphnia magna* | LC50 of 224.15 µg/L after 2 days exposure. | (Czub, Nawała, Popiel, Dziedzic, *et al.*, 2020) |
| 2-Chlorovinylarsonic acid | Bacteria | *Allivibrio fischeri* | EC50 of 0.031 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |
| Clark I | Crustacean | *Daphnia magna* | Significantly impairment on fecundity and somatic growth rate at 5 µg/L. | (Brzeziński *et al.*, 2020) |
| | Crustacean | *Daphnia magna* | LC50 of 0.037 mg/L after 2 days exposure. | (Czub, Nawała, Popiel, Brzeziński, *et al.*, 2020) |
| Diphenylarsinic acid | Bacteria | *Allivibrio fischeri* | EC50 of 124 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |
| | Crustacean | *Daphnia magna* | NOEC of 99.06 mg/L after 2 days exposure. | (Czub, Nawała, Popiel, Brzeziński, *et al.*, 2020) |
| Phenylarsonic acid | Bacteria | *Allivibrio fischeri* | EC50 of 97.1 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |
| | Crustacean | *Daphnia magna* | NOEC of 99.55 mg/L after 2 days exposure. | (Czub, Nawała, Popiel, Brzeziński, *et al.*, 2020) |
| Phenarsazinic acid | Bacteria | *Allivibrio fischeri* | EC50 of 5.33 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |
| | Crustacean | *Daphnia magna* | NOEC of 99.23 mg/L after 2 days exposure. | (Czub, Nawała, Popiel, Brzeziński, *et al.*, 2020) |
| Sulphur mustard | Crustacean | *Daphnia magna* | LC50 of 9.67 mg/L after 2 days exposure. | (Czub, Nawała, Popiel, Dziedzic, *et al.*, 2020) |
| Triphenylarsine | Bacteria | *Allivibrio fischeri* | EC50 of >200 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |
| | Crustacean | *Daphnia magna* | LC50 of 3.81 mg/L after 2 days exposure. | (Czub, Nawała, Popiel, Brzeziński, *et al.*, 2020) |

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|---|---|---|---|---|
| Triphenylarsine oxide | Bacteria | *Allivibrio fischeri* | EC50 of 155 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |
| | Crustacean | *Daphnia magna* | NOEC of 101.82 mg/L after 2 days exposure. | (Czub, Nawała, Popiel, Brzeziński, *et al.*, 2020) |
| Thiodiglycol sulfoxide | Bacteria | *Allivibrio fischeri* | EC50 of >74,250 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |
| | Crustacean | *Daphnia magna* | NOEC of 98.4 mg/L after 2 days exposure. | (Czub, Nawała, Popiel, Dziedzic, *et al.*, 2020) |
| Thiodiglycolic acid | Bacteria | *Allivibrio fischeri* | EC50 of 22.5 mg/L for bioluminescence inhibition test, Microtox™. | (Christensen *et al.*, 2016) |

Contrary to the relatively abundant toxicity data available for conventional explosives, data for CWAs and related chemicals is extremely scarce and do not provide sufficient information on the toxic effects these compounds may exert in aquatic organisms. For the taxonomic groups considered here, only toxicity test results using bacteria are widely available for these compounds. Additionally three studies evaluating CWAs using the crustacean *Daphnia magna* (Brzeziński *et al.*, 2020; Czub, Nawała, Popiel, Brzeziński, *et al.*, 2020; Czub, Nawała, Popiel, Dziedzic, *et al.*, 2020) were also retrieved (Table 6). However, no data was found for bis(2-chlorovinyl)arsinic acid (CASRN 157184-21-9) and bis(diphenylarsinyl) oxide (CASRN 2215-16-9), further illustrating the existent knowledge gap. The currently available data suggests that, as for the conventional explosives, also CWAs tend to exert lethal toxicity at concentrations ranging low mg/L. Here again, the collected human in vitro toxicity data suggests that the use of traditional endpoints may lead to an underestimation of the toxic potential of the tested compounds.

A few studies contribution to the possible hazards/risks of CWAs were recently published. Niemikoski et al. 2020, for instance, recently studied the metabolism of CWAs *in vitro* in cod

liver. They demonstrated that all examined compounds undergo biotransformation reactions initiated by the cod liver enzymes, except for triphenylarsine oxide, for which a different pathway was assumed. Such findings offer important insights into the largely unknown fate, behavior and toxicological impact of CWAs on aquatic organisms and humans (as fish consumers) (Niemikoski, Koske, *et al.*, 2020). Further, field studies have reported genotoxic and cytotoxic effects in different fish species collected from chemical munition dumping zones in the Baltic Sea (Baršiene *et al.*, 2014, 2016) and in the Southern Adriatic Sea (della Torre *et al.*, 2013). Straumer et al. 2020 used liver histopathology (Straumer *et al.*, 2020) and Ahvo et al. 2020 used biochemical biomarkers (Ahvo *et al.*, 2020) of the Atlantic hagfish (*Myxine glutinosa*) as bioindicators of the effects of CWAs. Further, following the concept previously described for CMCs, caged mussels have also been suggested as multi-biomarker species of dumped chemical weapons (Lastumäki *et al.*, 2020). Finally, trace concentrations of chemical warfare agent related phenylarsenic compounds were detected in Atlantic cod (*Gadus morhua*), witchflounder (*Glyptocephalus cynoglossus*), Norway pout (*Trisopterus esmarkii*), saithe *(Pollachius virens*) and lobster (*Nephrops norvegicus*) collected from Måseskär, a dumpsite near the Swedish coast and cod (*Gadus morhua*) collected from the Bornholm Basin, in the Baltic Sea, hence indicating the potential bioaccumulation of CWA-related chemicals in marine biota (Niemikoski, Söderström and Vanninen, 2017; Niemikoski, Straumer, *et al.*, 2020).

Despite the limitation of the currently available (eco)toxicity data, attempts were made to assess the environmental risk posed by CWAs to fish and fish communities, with particular focus in the in the Baltic Sea. Baršienė et al. (2014) assessed the environmental genotoxicity risk in flounder (*Platichthys flesus*) , herring (*Clupea harengus*) and cod (*Gadus morhua*) individuals collected at 42 different stations located in the Bornhom Basin, in the Baltic Sea. For that, the frequency in occurrence of micronuclei, nuclear buds and nucleoplasmic bridges in erythrocytes was used as genotoxicity endpoint and the risk determined based on calculated background

519 levels. Results show that reference levels of genotoxicity were not detected in any of the
520 samples stations. However, extremely high genotoxicity levels were estimated for 32 of the 42
521 stations samples between 2010 and 2012 (Baršiene *et al.*, 2014). On the fish community level,
522 however, Sanderson et al. (2014) suggest a negligible acute CWA risk quotient for the different
523 studied locations along the Nord Stream pipeline, in the vicinity of the Bornholm Deep. There,
524 CWA and related chemicals concentrations measured in environmental samples, both water and
525 sediment, were used to estimate predicted environmental concentration (PECs) in the different
526 locations. Further, given the absence of experimental data on the toxicity of CWA&RC to
527 aquatic organisms, including fish (the first experimental toxicity data was only published in
528 2016 and addressed effects in *Allivibrio fischeri*, see Table 6), predicted toxicity values for
529 individual CWAs were used to derive an acute sensitivity distribution (SSD) for 12 fish species
530 (HC5=290 µg/L), here representing the concentration where 95% of the acute LC50 of the fish
531 species in the community is not exceeded without an assessment factor. Hence, the estimated
532 risk quotients result from the quotient of the PEC by the derived HC5 (Sanderson *et al.*, 2014).

533

534 **5. Future Perspectives and Research Needs**

535 The limitations of the currently applied analytical methods were previous discussed above
536 (section "Detection of munition constituents in the environment"). Current sampling
537 techniques predominantly rely on conventional grab sampling using of Niskin bottles to take
538 water samples at a pre-determined depths (Öllers *et al.*, 2001; Arpin-Pont *et al.*, 2016).
539 However, this type of active sampling presents some drawbacks. Specifically, the presence of
540 munition constituents at trace levels requires large volumes of water to reach the required
541 analytical sensitivity. Further, such techniques only provide a snapshot of the environment at
542 the specific time of the sampling (Vrana *et al.*, 2005). Passive sampling devices, however, have
543 emerged as a promising tool. These devices contain a sorption medium able to capture

544 pollutants by simple diffusion, driven by a difference in chemical potentials of the analyte
545 between the receiving phase and the external environment (Górecki and Namienik, 2002;
546 Chimuka, Cukrowska and Tutu, 2008). Passive samplers (1) require reduced effort for
547 deployment, retrieval and sample processing and allows prolonged storage under cooled
548 conditions before analysis, (2) can detect episodic events giving a more representative picture
549 of the contaminants present in the water column, and (3) can detect ultra-trace contaminant
550 concentrations due to the ability to sample large water volumes and in situ pre-concentrations,
551 resulting in increased method sensitivity (Vanryckeghem, 2020). Passive sampling also allows
552 transfer of environmentally realistic contaminant mixtures into biotest system either by passive
553 dosing or extract spiking (Moeris *et al.*, 2019). However, the use of passive samplers also
554 presents some challenges such as the need for extensive calibration studies prior to field
555 deployment, difficulties in inter-study comparisons due to different procedures during sampler
556 preparation, handling, storage and calibration or the fact that the used membranes can
557 significantly reduce the sampling rates making impossible the detection of the accumulated
558 compounds via analytical methods (Vanryckeghem, 2020). Furthermore, different projects
559 have recently been focusing on the development and optimization on both *in situ* and *ex situ*
560 techniques for the detection of munition-related chemicals present in marine munition
561 dumpsites. For instance, the ExPloTect project specifically aims at developing a sea-going
562 device ultimately enabling the *ex situ* near-real-time detection of multiple munition-related
563 chemicals present in the water column of marine dumpsites (https://www.explotect.eu/de).
564 Similarly, within AMMOTRAce, new laser photoionisation mass spectrometry and ion
565 mobility spectrometry based approaches will be developed and integrated with a membrane
566 inlet system in a remotely operated vehicle for the in situ detection of munition-related
567 chemicals (https://www.geomar.de/en/ammotrace).

In addition to the discussed improvements to the applied analytical and detection methods, it is of utmost importance to further understand the impact of munition constituents leaking from corroded shells on human health and the environment. The summary presented above (in section "Adverse effects of munition constituents to environmental and human health") demonstrates the need to generate new data aiming at closing the existent knowledge gap on the toxicity of chemicals warfare agents and renew the outdated data currently available for conventional munition constituents. For that, it is essential to study the effects of short- and long-term exposure of the mentioned chemicals in relevant species from different trophic levels, including bacteria, algae, copepods and fish. Ideally, such studies should follow previously established standard protocols (e.g. by either the International Organization for Standardization (ISO) or the Organization for Economic Co-operation and Development (OECD)), to ensure the repeatability and reproducibility of the results.

In fact, the use of *in vitro* assays, using either human or fish cell lines, allows the study of a wide variety of endpoints while eliminating ethical concerns related to animal cruelty or human testing, and reducing chemical, water and material waste. Among the many potential *in vitro* endpoints are cell viability (Dayeh *et al.*, 2004; Rudzok *et al.*, 2009) and cell proliferation (Stadnicka-Michalak, Schirmer and Ashauer, 2015), chemical toxicokinetics and toxicodynamics (Stadnicka, Schirmer and Ashauer, 2012; Stadnicka-Michalak *et al.*, 2014; Chang *et al.*, 2018), chemical bioaccumulation and biotransformation (Saunders *et al.*, 2018; Stadnicka-Michalak *et al.*, 2018) and the mechanisms behind mitochondrial toxicity, possible via the use of Seahorse Analyzer (Müller *et al.*, 2019). In fact, in a recent study, Niemikoski et al. (2021) exemplified applicability of *in vitro* assays to gain knowledge on the potential impact of munition-related chemicals by assessing the cytotoxicity and metabolism of diphenylarsinic acid its major metabolite, diphenylarsine glutathione conjugate, in the rainbow trout liver cell

line, RTL-W1, with the results showing that diphenylarsine glutathione conjugate is two orders of magnitude more toxic than diphenylarsinic acid (Niemikoski *et al.*, 2021).

As an alternative to both *in vivo* and *in vitro* testing, extensive attention has been given to the development and optimization of *in silico* approaches such as the quantitative structure-activity relationships (QSARs) models. In summary, QSARs have been developed to predict the toxicity of a given chemical in an organism-based toxicity data previously generated for chemicals with similar physicochemical properties. Over the last few years, such models have been applied in various contexts to predict the effects of chemicals in human an environmental health. For instance, Jia et al. used a QSAR model to predict the toxicity of various pesticides in rainbow trout (Jia *et al.*, 2020) while Klüver et al. developed a baseline toxicity QSAR model for fish embryo acute toxicity test with zebra fish embryo (Klüver *et al.*, 2016). Additionally, QSAR models have been used to predict the effects of endocrine disrupting chemicals on human health (Heo, Safder and Yoo, 2019), the carcinogenicity of PAHs and its transformation products (Gbeddy *et al.*, 2020) or biological activity of nanoparticles (Burello and Worth, 2011; Singh and Gupta, 2014). Moreover, to further support the proper application of QSAR models, the OECD made available a QSAR Toolbox, accompanied by introductory guidelines and examples - https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm, which was incorporated by the European Chemicals Agency (ECHA) - https://echa.europa.eu/support/oecd-qsar-toolbox. A similar toolbox compiling various QSAR models was created by the Danish Environmental Protection Agency - https://qsarmodels.food.dtu.dk/.

Finally, even though the testing of chemicals' toxicity under laboratorial conditions is essential to acquire information of its baseline toxicity, such tests merely provide a simplistic representation of the complex interactions taking place in the environment. In the specific case of munition dumpsites, it is of extreme relevance to understand the effects of presence of such

long-lasting stressors on the biodiversity of the impacted locations. To that end, the emergence of the environmental DNA (eDNA) significantly increases the ability to detect and quantify biodiversity hence providing a more realistic overview of the ecological status of the study site. In short, eDNA comprehends genetic material originating from the hair, skin, urine, feces, gametes or carcasses of organisms present in water, soil or sediment (Thomsen and Willerslev, 2015). Among its advantages, when compared to other techniques, are the fact that eDNA is fast, efficient, relatively cheap (Ficetola, Manenti and Taberlet, 2019; Sales *et al.*, 2020), non-destructive and non-invasive (Moran, Prosser and Moran, 2019; Leempoel, Hebert and Hadly, 2020), allows the early detection of biological invasions as well as an accurate identification of target organisms (Nardi *et al.*, 2019; Schumer *et al.*, 2019) and offers a broad taxonomic breadth thus allowing simultaneous biodiversity assessment for a wide range of organisms (Thomsen and Sigsgaard, 2019; Zhang *et al.*, 2020). Despite its wide applicability and multiple advantages over traditional surveys, eDNA presents limitations that should be considered. Among them, its limited appropriacy for studies where information on abundance or biomass of species, its ecology or its conservation status are required (Baldigo *et al.*, 2017; Trebitz *et al.*, 2017), its species- or taxa-specific performance (Olds *et al.*, 2016; Yamamoto *et al.*, 2017; Rose *et al.*, 2019), and high impact of environmental conditions on eDNA degradation, particularly in warm and humid conditions (Harrison, Sunday and Rogers, 2019; Sirois and Buckley, 2019), hence making its applicability context-dependent. Beng and Corlett recently published a comprehensive review covering the wide applications, advantages and limitations of eDNA thus clarifying the potential as well as the drawbacks of the mentioned technique (Beng and Corlett, 2020).

## Conclusion

The environmental impact of the dumpsites resulting from World War I and World War II has been overlooked for decades. Countries directly involved in the conflicts tend to be more impacted by munition dumpsites. The growing interest in the exploration and development of blue economy has urged the need to further understand the behavior of the dumped munition in marine systems as well as their interaction with humans and the marine wildlife. This article utilized a systematic literature review to summarize the current knowledge on detection environmental concentrations of chemicals related to dumped munition and well as their impact on human and environmental health. Despite the existing studies on the effects of munition constituents, with particular focus on conventional explosives and their metabolic products, evidence gathered in this review unravels the poor understanding of the toxic potential of such chemicals at lethal and specially sub-lethal endpoints. To overcome such knowledge gap, different research approaches have been suggested aiming at generating new, accurate and repeatable data useful for adequate risk assessment.

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