DAIMON ECOTOX TOOLBOX

Toolbox for the assessment of marine munitions' impact on biota

With this modular Toolbox we provide a strategy and methodological tools to answer the question whether the marine ecosystem might be affected by dumped munitions and potentially hazardous chemical munitions components. The Toolbox is designed for use in monitoring and assessment as well as supporting decision making.

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with contributions from many other DAiMON Project Partners (see Table 1)

Content

1. DAIMON EcoTox Toolbox in a nut shell

The DAIMON Ecotox Toolbox is an output of the Interreg project DAIMON (Decision Aid for Marine Munitions, 2016-2019). It provides tools (concept, strategy, methodological recommendations and guidelines) for analyzing and assessing exposure and ecological risks associated with sea-dumped chemical and conventional munitions and potentially hazardous warfare agents emitted from the munitions. It is designed to address the following hypothetical cases and to help answering associated questions:

> *For a given geographical maritime area there is either information available or suspicion that dumping of munitions and/or warfare agents took place in the past and the questions arise whether these munitions/warfare agents may pose a risk to marine organisms in their habitat and whether measures to minimize or avoid ecological risks have to be taken[1].*

Answers to these questions can be given by using the Toolbox, and this may lead to decisions and, ultimately, the implementation of measures to cope with ecological risks identified.

The strategy for application of the Toolbox is based on a two-steps-concept: a Screening Study (*Step 1*) and a following Detailed Study (*Step 2*) as required. Both steps comprise a set of methodological approaches and techniques to be applied, for which recommendations and guidelines are given (as Toolbox Fact Sheets). These either provide detailed instructions for the practical application of methods or, if appropriate guidelines have been published elsewhere, short description and references to published established instruction. Further on, the toolbox gives guidance on how to interpret the results and how to assess risks.

The toolbox components reflect the methodological development achieved through the DAIMON project as well as through its predecessor projects (CHEMSEA, MODUM) and address the following aspects (see Fig. 2):

- Munitions detection and identification
- Analysis of hazardous substances
- Analysis of biological effects
- Other approaches
- Data analysis and assessment
- Decision support

A Screening Study is conducted as a first step to assess possible ecological risks associated with dumped munitions in relatively short time. It has a limited number of general and specific easy-to-apply components and focuses on measurements on the presence of dumped munitions, the release of potentially harmful substances into the environment and on biological effects of these substances in wild organisms.

[1] Such cases are not purely theoretical, but realistic scenarios, because there is an apparent lack of information on ecological risks associated with known munition in the sea and because it is well known that not all dumping operations, e.g. those occurring at the end and after WW II, have been properly documented.

If the results of the Screening Study do not sufficiently meet the requirements for an appropriate risks assessment, the following step, a Detailed Study, can be launched, which will provide more specific information on ecological risks and will generate more comprehensive information for risks assessment, decisions and management actions. The Detailed Study comprises a larger set of components and parameters than the Screening Study. The focus is again on chemical analysis of potentially harmful substances as well as *in situ* biological effects studies, but may also include other approaches such as lab or field exposure studies and toxicity assays. The Detailed Study, thus, includes more specific methods which can provide a more detailed description of the ecological risks.

Based on the results of the Screening and/or the Detailed Study, a risk assessment can be performed and, again based on the results, decisions can be taken and management actions can be implemented as appropriate.

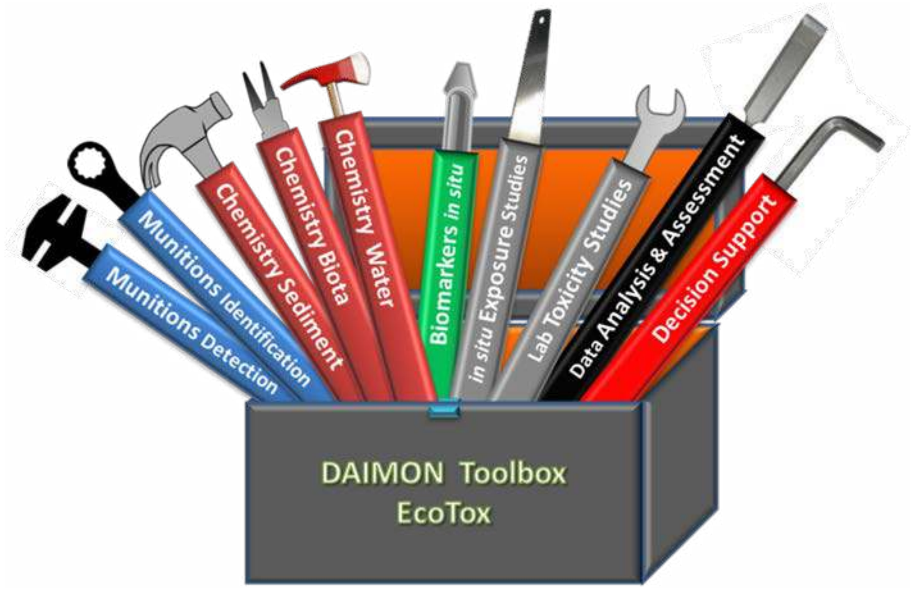

Figure 1: Components of the DAIMON EcoTox Toolbox

2. Background of the Problem

Millions of tons of conventional and chemical munitions and warfare agents are lying on the bottom of the world's oceans, partly in deep sea areas, but partly also in shallow coastal areas. A large part are legacies of World Wars I and II and originate, e.g., from military operations, including aerial attacks, war at sea, scuttling of battle ships as well as from intentional disposal of munitions and warfare agents at the end and after the end of the wars. However, military operations and munitions production and use have continued since then and, thus, disposal of munitions and warfare agents into the seas did not stop. In fact, sea dumping of obsolete or outdated munitions was a common practice in a number of European and non-European countries until the 1970s. Hundreds of dumping areas are either known or are suspected to exist on a global scale (Beldowski et al. 2016, 2018). It is estimated that in total one Mio. t of chemical munitions has been dumped into seas and oceans and that the amount of chemical munitions In European waters is almost 700.000 t (Arison 2013).

In the Baltic Sea and in the Skagerrak, large amounts of chemical munitions and warfare agents, mainly from German production and stockpile sites, were dumped after the end of World War II by order of the allied forces (Knobloch et al. 2013). In the deep waters of the Skagerrak, more than 30 ships were scuttled, loaded with approx. 200,000 t of chemical munitions. In the Bornholm Basin and Gotland Deep, estimated 42,000 to and 65,000 t of chemical munitions were dumped after the end of World War II. But also shallow areas like the Little Belt were used as dumpsites of chemical munitions. Chemical warfare agents dumped were mainly sulfur mustard, Clark I/II, Adamsite, Lewisite, Tabun and Phosgene.

The majority of munitions in the sea is classified as conventional munitions, mostly high-explosive munitions (filled with explosives such as TNT) but also incendiary bombs (e.g. filled with white phosphorus). Conventional munitions are spread over wide areas of the Baltic Sea and the North Sea, thus they can be found also close to the shores. In German waters of the North Sea and Baltic Sea, approximately 1.6 Mio t of munitions are still lying on the sea floor, partly in areas close to the shore, the utmost majority of which is conventional munitions.

Only recently, the problematics associated with marine munitions aroused the interest of the public, politics, and science. This has been primarily triggered by the construction of offshore wind parks as well as laying of cables and pipelines on the sea ground. Frequently, these activities lead to discoveries of explosive ammunition.

In addition to security issues, it is conceivable that this massive amount of munition dumped at sea negatively impacts the marine environment and its biota. This calls for action to take measures to recover or delaborate marine munitions. The following issues require particular attention: What is the scale of this contamination? What are the possible long-term effects of progressing corrosion of ammunition and the associated release of toxic substances? To what extend are humans and marine ecosystems under threat? How do we manage marine munitions?

The DAIMON project targeted the ecological risk assessment of marine munitions on fish by combining field studies and laboratory experiments. The ultimate goal of DAIMON was to provide decision support for dealing with dumped munitions. One part to enable decision maker to perform a risk assessment is the here presented DAIMON Ecotox Toolbox. It will help to answer the question whether dumped munition/warfare agents in a given geographical area may pose a risk to marine organisms in their habitat.

3. How the DAIMON EcoTox Toolbox can help: the concept

The DAIMON project and its predecessor projects have clearly demonstrated that sea-dumped munitions may pose ecological risks to organisms inhabiting geographical regions affected. There is now evidence that hazardous substances originating from chemical warfare agents as well as from explosives and other chemical warfare components are emitted from corroded munitions or disposed containers with warfare agents and are taken up by wild organisms or experimentally exposed organisms (Niemikoski et al. 2017, unpublished DAIMON results). There is also indication that these substances may cause biological effects such as biochemical responses as well as genotoxic or carcinogenic effects and, if concentration exceed crtitical levels, increased mortality (…).

However, from experience made in the projects it became clear that for many regions known to be affected by dumped munitions information on the extent of ecological risks and effects is largely lacking. Furthermore, there are many regions where the presence of munitions has so far only been suspected but not yet verified. Especially for the latter areas, basic studies on the amount and types of munitions present are required before any ecological risk assessment can be made.

The DAIMON project also provided evidence through method testing and validation as well as through pilot studies in various munitions dumpsites that studies on ecological risks have to be based on an integrated approach, encompassing a set of physical, chemical and biological methods. Depending on the specific problem to be addressed, these need to be applied in a combined manner, following a clear concept and strategy as well as by utilizing documented technical guidelines.

Here, the DAIMON EcoTox Toolbox comes into play, which was developed as one of the main outcomes of the DAIMON project. It constitutes a joint effort of Project Partners and Associated Organisations contributing to DAIMON Activity 2.5 "Assessment of Marine Munitions' Impact on Biota". Its primary goal is to provide strategic considerations, recommendations for appropriate methods to analyse and assess ecological risks of dumped munitions to marine biota as well as technical guidelines for methods addressed (see Figure 1 and Fact Sheet Table 1).

4. DAIMON EcoTox Toolbox strategy

The application of the Toolbox is based on two pathways: (1) a Screening Study and (2) a Detailed Study (see Fig. 2). The screening study constitutes the first step and is followed by a Detailed Study if the results of the Screening Study do not allow a proper risk assessment.

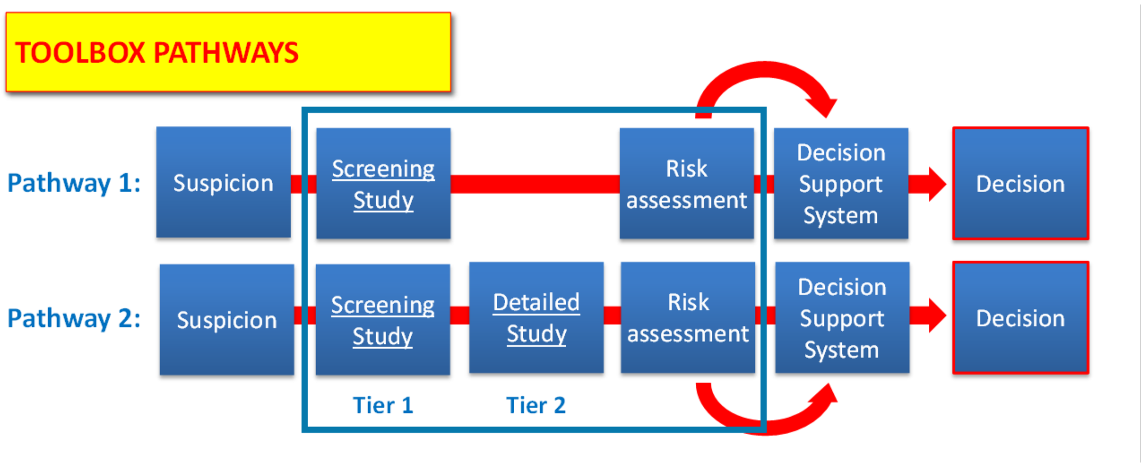

Figure 2: The two toolbox pathways (for components in the box, Fact Sheets are available providing methodological guidelines)

4.1. Step 1: Screening Study

The Screening study is the first step in the process of biota impact assessment. It is designed to provide information on three principal components:

(1) presence of munitions
(2) release of potentially hazardous agents into the environment and
(3) biological effects of agents on biota in the region

From the methods available in the Toolbox (see Fact Sheet Table 1), a limited number of physical, chemical and biological methods is selected that are robust and relatively easy to apply and that provide answers to each of the three above components. The basic chronology of conducting a Screening Study is illustrated in Fig. 3a and 3b.

Depending on the amount and type of information available prior to applying the Toolbox in a Screening Study, not all three components will have to be addressed. For instance, if studies are to be focused on a dumpsite with known munitions objects with, however, knowledge neither on chemical emissions nor on biological effects, only components (2) and (3) will be addressed. If, in contrast, there is only a suspect that munitions may be present and may pose ecological risks, all three components will have to be addressed in a chronological order.

In the latter case, the first task in the Screening Study will be to check if data are already available that may facilitate a proper risk assessment. Data sources to be explored are, e.g., military or civil archives, potentially documenting and mapping past dumping operations[2]. Furthermore, data from regular chemical monitoring programs could be examined for information linked to dumping of munitions and the emission of hazardous substances from corroded munitions, resp. For instance, total arsenic (As) and mercury (Hg) are measured in many chemical monitoring programmes, mainly in sediment and/or biota, since both enter the marine environment from various sources and are considered as ecotoxic and as anthropogenic pollutants affecting safety of food from the sea for human consumers. Both metals are also constituents of dumped munitions, As as basis of some chemical warfare agents (e.g. Clark I and II, Adamsite, Lewisite) and Hg as chemical basis of mercury fulminate used as explosive primer (Beldowski et al. 2019).

If no archived data are available, the next step in the Screening Study is to apply methods aiming at detecting and identifying munitions on the seafloor, e.g. applying hydroacoustic and magnetometric measurements (see Fact Sheet Table 1, Fact Sheets 1.1-1.3).

If no munitions objects were found during the physical examination of the seafloor and if, thus, the area is considered free of munitions, not further steps need to be taken. However, if munitions was found and the type of munitions characterized (chemical munitions or conventional munitions), chemical screening of hazardous substances and measurements on in situ biological should follow, ideally carried out in parallel. For the Screening Study, it is recommended to restrict chemical screening on measurement of a small number of sediment samples taken within and around the dumpsite, preferably covering a contamination gradient. Depending on the type of munitions detected and identified, chemical analysis is targeted to relevant explosives (e.g. TNT and key

[2] See Annex xx

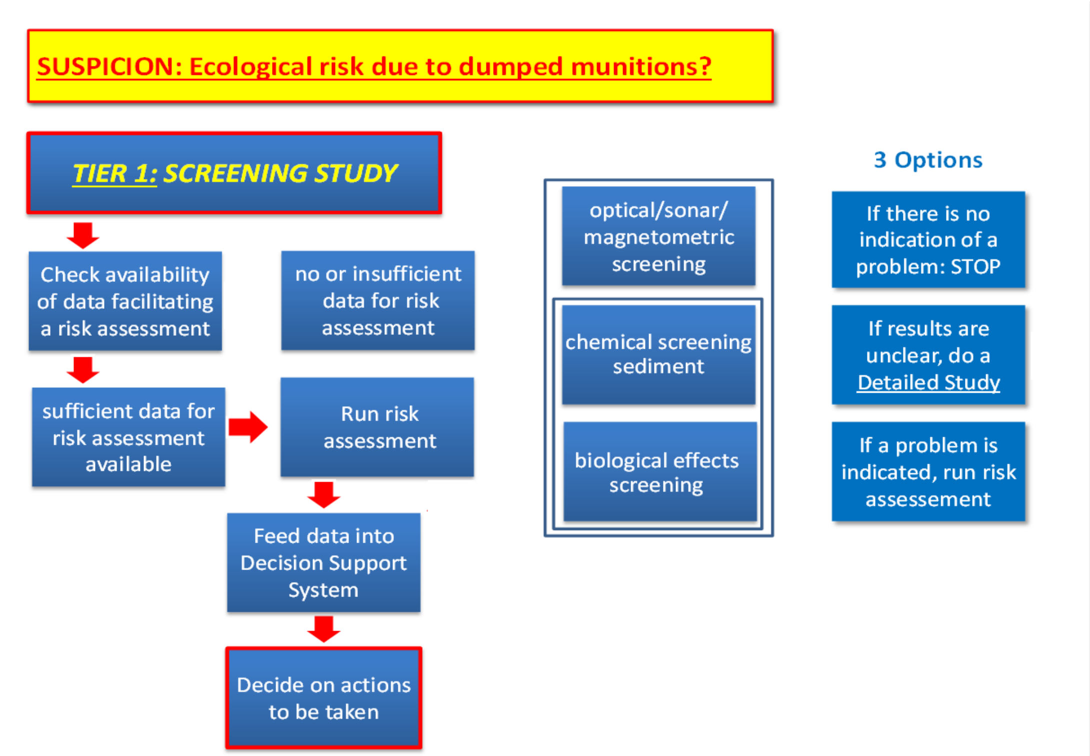

Figure 3a: Screening Study: Search for information available to do assessment based on existing data

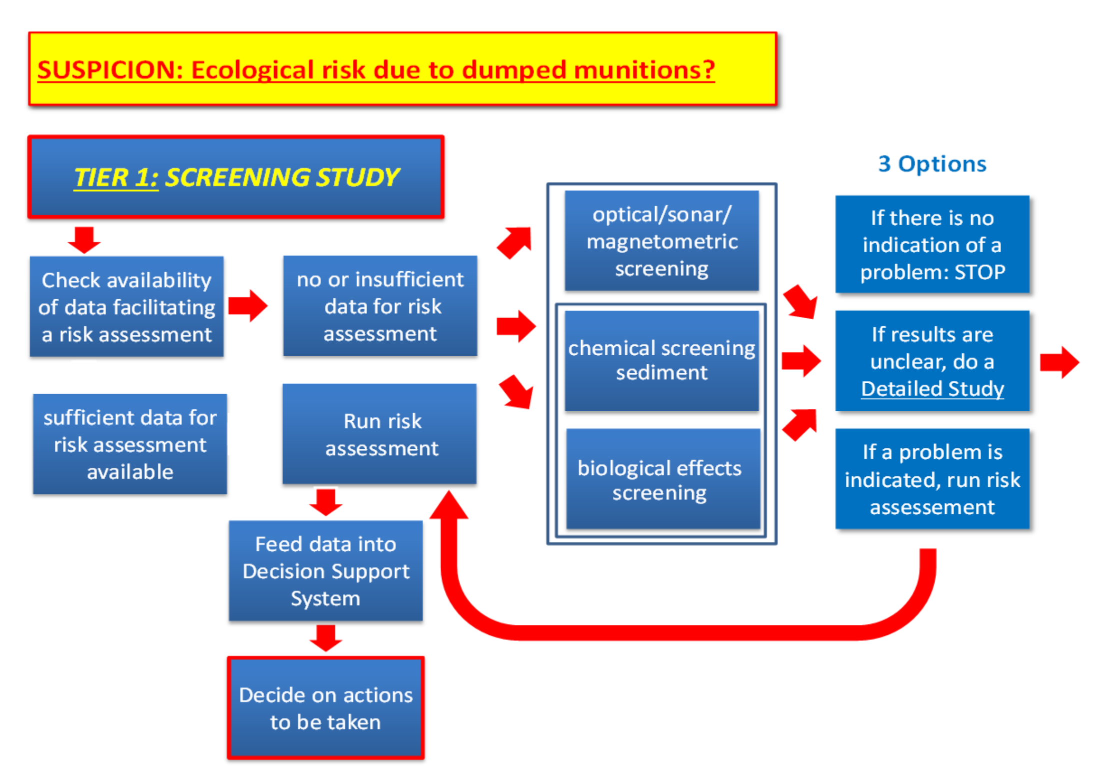

Figure 3b: Screening Study: If no information is available to do assessment based on existing data, screening (optical/sonar/magnetometric, chemical, biological) is carried out

metabolites of the ADNT group) or to the most common key chemical warfare agents (arsenic compounds and oxidation products, resp., or compounds related to sulfur mustard) (see Fact Sheet Table 1, Fact Sheets 2.1.-2.3).

Biological effects measurements in the Screening Study should be carried out using a selected set of biomarkers, applied on target species inhabiting the area of interest, e.g. wild fish or bivalves. For both taxonomic groups, useful biomarkers were identified during the DAIMON project (Fact Sheet Table 1, Fact Sheets 3.1-3.27). It is recommended to select and analyse three *in situ* biomarkers the selection of which is based on the problem (for screening bioeffects of explosives, other biomarkers may be used than for CWA). Sampling should be conducted according to recommendations for biota sampling (see Fact Sheet 3.1)

If the results of measurements addressing all three components of the Screening Study (see above) are positive and, thus, an ecological risk is strongly indicated, a formal risk assessment (which may not be needed in all cases) can be run and the results can be incorporated in the DAIMON Decision Support System (DSS)[3]. Ultimately, the outcome of this process may lead to management measures aiming at minimizing or avoiding ecological risks and damage.

If none of the components indicate a problem, no further actions are required. However, if for instance munitions was found and identified, but findings regarding sediment contamination and bioeffects were negative, an appropriate management measure would, e.g., be to implement a monitoring/surveillance programme.

4.2. Step 2: Detailed Study

If the results of the Screening Study (Step 1 of the Toolbox) are considered not sufficient to perform a risk assessment, it is recommended to add a Detailed Study, consisting of (1) alternative methods to detect munition (2) more specific chemical measurements in different matrices (water, sediment, biota) and/or of munitions compounds other than those measured during the screening study, (3) more specific biological effects measurements analysing other biological endpoints and/or target organisms than those used during the screening study. Furthermore, (4) other approaches may be applied, e.g. sediment toxicity bioassays, experimental *in vitro* or *in vivo* toxicity assays, using suitable target cell/tissue/organism models (see Fact Sheets 4.3-4.6). Another appropriate option may be to carry out *in situ* cage exposure experiments with ecologically relevant test species (e.g. fish and/or bivalves) (see Fact Sheet 4.1.-4.2). The components of the Detailed Study depend on the results of the screening study and of the question to be answered. The general strategy for conducting the Detailed Study is given in Fig.2.

[3] The Decision Support Sytem (DSS) is a major output of the DAIMON project and facilitates decisions on measures to be taken to minimize risks to different protection goods. The DSS can be seen as the final step of the EcoTox Toolbox).

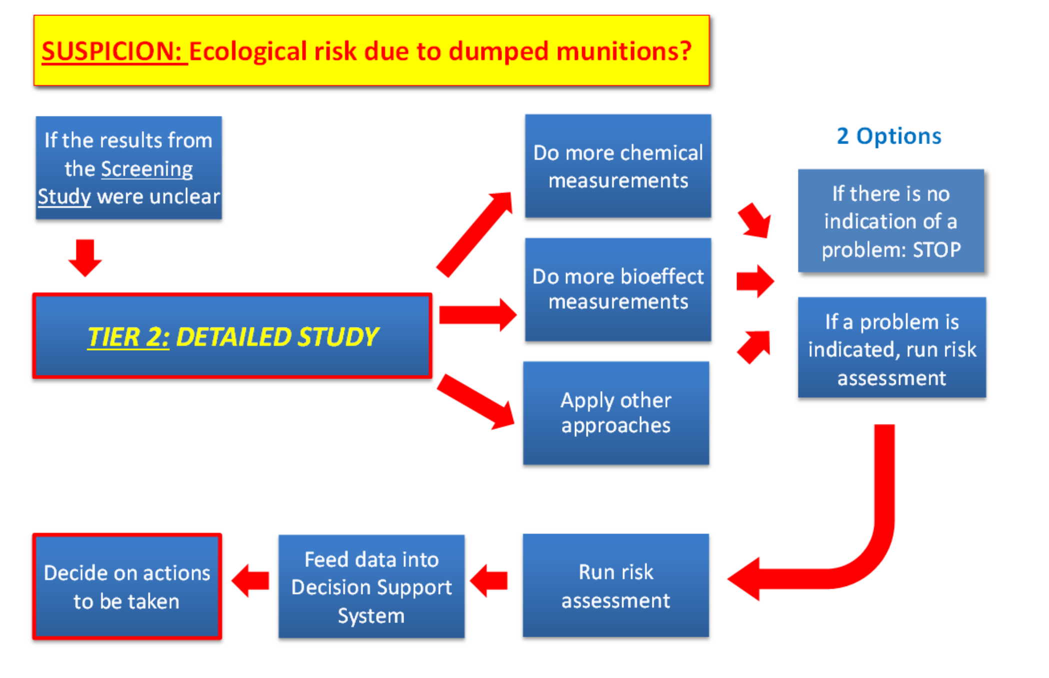

Figure 4: Schematic illustration of the Detailed Study

5. Tools in the box

In the following, methods recommended for the components of the Screening Study (Step 1) and the Detailed Study (Step 2) are described for an overview. Detailed descriptions including references can be found in the Fact Sheets (Annex).

The methodological descriptions are grouped into 4 assessment categories: (1) Munitions detection and identification, (2) Hazardous substances, (3) Biological effects and (4) Other approaches (see Fact Sheet Table 1). Category (1) comprises hydroacoustic, magnetometric, visual and chemical methods (Fact Sheets 1.1-1.3), category (2) mainly chemical methods to measure CWA or explosives in sediment, water and biota (Fact Sheets 2.1-2.11), category (3) biological methods (biomarkers) to analyse effects of hazardous substances on fish or mussels, category (4) methods to be used for lab-based toxicity studies or in situ exposure experiments (Facts Sheets 4.1-4.6).

For each of the methods, a Fact Sheet was prepared by DAIMON project Partners (see Fact Sheet Table 1). The structure and layout of the Fact Sheets was always the same, however, the degree of information detail varied. If methods have been described elsewhere in the literature, the Fact Sheets are kept short and include only the key points required to understand the method and references to relevant publications. If the method has not been properly documented, the Fact Sheets provide more detail.

Tab. 1: Components of the DAIMON EcoTox Toolbox - Fact Sheets

Assessment category	Toolbox component	Parameter	Matrix	Toolbox Fact Sheet Title	Authors
1: Munitions detection and identification	Munition detection	Side scan sonar (AUV)	Sediment surface, Subbottom	1.1: Munition detection procedure with a hydroacoustic and magnetometry equipment	M. Grabowski
	Munitions identification	Camera systems (ROV, optical, acoustic)	Sediment surface, subbottom	1.2: Identification and visual inspection of detected munitions-like objects	M. Grabowski
	Munitions identification	Neutron activation analysis	Munitions	1.3: Munitions identification via Neutron Activation Analysis (NAA)	H. Vainionpaa
2: Hazardous substances	Sediment chemistry	CWA and degradation products/metabolites	Sediment	2.1: Chemical analysis of CWA-related compounds in sediment with LC-MS/MS	H. Niemikoski , H. Lignell
				2.2: Chemical analysis of CWAs and degradation products in sediment with GC-MS/MS	H. Lignell
		TNT and degradation products/metabolites	Sediment	2.3: Chemical analysis of conventional munitions in sediment with GC-MS/MS	H. Lignell
	Water chemistry	CWA and degradation products/metabolites	Water	2.4: Chemical analysis of CWA-related compounds in pore water with LC-MS/MS	H. Niemikoski , H. Lignell
	Biota chemistry	Biota sampling	Fish/Mussels	see Fact Sheet 3.1 for wild fish	T. Lang/-
		CWA and degradation products/metabolites	Fish bile	2.5: Chemical analysis of CWA-related phenylarsenic chemicals in bile	H. Niemikoski
			Fish muscle	2.6: Chemical analysis of CWA-related phenylarsenic chemicals in cut fillet	H. Niemikoski
			Fish liver	2.7: Chemical analysis of CWA-related phenylarsenic chemicals in fish liver	H. Niemikoski
			Fish gills	2.8: Chemical analysis of CWA-related phenylarsenic chemicals in fish gills	H. Niemikoski
			Mussel tissue	2.9: Chemical analysis of CWA-related phenylarsenic chemicals in mussel soft tissue	H. Niemikoski
		TNT and degradation products/metabolites	Fish bile	2.10: Extraction of explosives and metabolites from fish bile	N. Goldenstein

			Fish bile	2.11: Analysis of explosives and metabolites via HPLC-QQQ-MS	N. Goldenstein
3: Biological effects	Biota sampling	Fish sampling	Wild fish	3.1: Sampling of wild fish	T. Lang
		Tissue homogenization for biomarker studies	Fish liver/Mussel digestive gland	3.3: Homogenisation of fish liver and mussel digestive gland tissues	A. Ahvo, K. Lehtonen, R. Turja
			Fish muscle/Mussel gill tissue	3.4: Homogenisation of fish muscle and mussel gill tissues	A. Ahvo, K. Lehtonen, R. Turja
	Fitness	Fulton's condition factor	Whole fish	3.5: Fulton's Condition Factor (CF) in Fish	T. Lang, K. Straumer
		Mussel condition factor	Whole mussel	3.6: Condition Index (CI)	M. Brenner
		Hepatosomatic index	Fish liver	3.7: Hepatosomatic Index (HSI) in Fish	T. Lang
		Mussel glycogen	Mussel digestive gland	3.8: Glycogen – accumulation of primary energy reserve in mussels	M. Brenner
	General stress	Blood glucose	Fish blood	3.9: Hematology – blood glucose level	K. Straumer, T. Lang
		Oxidative stress	Fish liver, mussel digestive gland	3.10: Lipid peroxidation	A. Ahvo, K. Lehtonen, R. Turja
			Fish liver, mussel digestive gland, mussel gill tissue	3.11: Superoxide dismutase activity	A. Ahvo, K. Lehtonen, R. Turja
			Fish liver, mussel digestive gland and gill tissue homogenates	3.12: Catalase activity	A. Ahvo, K. Lehtonen, R. Turja
			Fish liver and mussel digestive gland and gill tissue homogenates	3.13: Glutathione peroxidase activity	A. Ahvo, K. Lehtonen, R. Turja
			Fish liver and mussel digestive gland and gill tissue homogenates	3.14: Glutathione reductase	A. Ahvo, K. Lehtonen, R. Turja
		Macromolecular defense/xenobiotic metabolism	Fish liver tissue and mussel gill and digestive gland tissue homogenates	3.15: Glutathione S-transferase activity	A. Ahvo, K. Lehtonen, R. Turja

	Disease/ pathology	Gross diseases/parasites	Whole fish	3.16: Externally visible fish diseases (EVFD)	T. Lang, K. Straumer
		Histopathology	Fish liver	3.17: Fish liver histopathology	T. Lang, K. Straumer
		Lysosome membrane stability	Fish liver tissue, mussel digestive gland tissue, mussel haemocytes	3.18: Lysosome membrane stability	K. Lehtonen, A. Lastumäki, M. Brenner
		Lipofuscinosis	Mussel	3.19: Lipofuscinosis – pathological accumulation of lysosomal lipofuscin	M. Brenner
		Lipidosis	Blue mussel	3.20: Lipidosis – pathological accumulation of neutral lipids	M. Brenner
	Immunotoxicity	Haematology	Fish blood	3.21: Hematology - erythrocytes, hemoglobin, hematocrit and leucocrit	K. Straumer, T. Lang
		Differential blood cell count	Fish blood	3.22: Hematology - differential white blood cell count	K. Straumer, T. Lang
	Neurotoxicity	Acetylcholinesterase inhibition	Fish muscle and mussel gill tissue homogenates	3.24: Acetylcholinesterase inhibition	A. Ahvo, K. Lehtonen, R. Turja
	Carcinogenicity	Macroscopic liver neoplasms	Fish liver	3.25: Macroscopic liver neoplasms (MLN)	K. Straumer, T. Lang
	Genotoxicity	Micronucleus assay	Fish Blood	3.26: Micronucleus Assay (MN)	T. Lang
			Fish liver and mussel digestive gland tissue homogenates	3.27: Gene transcription	R. Turja, K. Lehtonen
4: Other approaches	*In situ* exposure studies		Mussel	4.1: The mussel caging approach	A. Lastumäki, K. Lehtonen
			Fish	4.2: The fish caging approach	T. Lang, K. Straumer
	Lab toxicity studies	Fish embryo assay	Zebrafish (*Danio rerio*)	4.3: Zebrafish embryo acute toxicity test (FET)	D. Koske
		Comet assay	Fish, e.g. zebrafish	4.4: Comet Assay (applied to zebrafish embryos)	D. Koske
		Mussel exposure	Blue mussels	4.5: Mussels lab exposure to warfare agents	M. Brenner
		Flatworm exposure	Flatworms	4.6: Fluorescence assay for the detection of the activity of ABC transporters induced by toxicants	U. Bickmeyer

6. How to select tools

In a full Screening Study addressing the presence of munitions as well as chemical and biological screening, the first step is to confirm the presence of munitions and identify the type of munitions (chemical or conventional) by applying methods detailed in Fact Sheets 1.1-1.3.

The selection of chemical tools depends on the type of munitions found: in case of chemical munitions, the sediment has to be screened for the presence of chemical warfare agents (CWA) and their degradation products, resp. (Fact Sheets 2.1., 2.2.), in case of conventional munitions, it is recommended to screen the sediment for TNT and its major degradation products/metabolites, resp. (Fact Sheet 2.3).

For biological effect screening, it is recommended to select three methods from Fact Sheet Table 1 (Fact Sheets 3.1.-3.27), including at least one method related to the respective target (explosives, CWA) if possible as well as general methods. The method selection should be closely related to the suspected problem regarding type of munition, toxic chemicals and species possibly affected.

To conduct a Detailed Study, there is no rigid design. However, in case the Detailed Study needs to deepen all aspects of the Screening Study, the principle is that additional methods selected from Fact Sheet Table 1 should be applied that provide data on the presence of hazardous substances and of biological effects of these substances. Chemical and biological analyses in the Detailed Study can be performed with a broader set of target indicators which need more technological background and sophisticated time-consuming strategies than those performed in the Screening Study. The method selection should take into account alternative parameters which could have been overlooked in the screening study.

7. How to apply tools and interpret the results

The Toolbox provides guidance how to select and apply methods and how to assess the results of their application. In the following, some scenarios are described. As a general principle, the application of a method to analyse the presence of munitions, of hazardous substances or of biological contaminant effects will lead to a “Yes” or a “No” result. The set of three biological effects techniques are regarded together. Their individual results are merged to a combined result. It is positive (“Yes”) when at least 2 out of 3 methods show a positive result. Otherwise the combined results of biological effects methods are classified as “No”. The toolbox application leads to an assessment of the situation and a recommendation regarding measures to be taken.

The first scenario describes a Screening Study and their results (see Figure 5). The starting point is the suspicions, that munitions may be present in a given region and that this munitions may pose a threat to organisms (in this case fish) in the habitat. In the first step, the presence of munitions was confirmed and munitions were identified as conventional munitions. In the second step, the presence of TNT-related hazardous substances in the sediment was confirmed by chemical analysis. For the biological screening, three methods were selected, one representing general stress-related effects (externally visible fish diseases), the second one carcinogenic effects (liver tumours) and the third one genotoxic effects (micronucleus assay). Since two of the three biomarkers responded, the fish were regarded as affected by hazardous substances originating by the dumped munitions found.

From the results of the scenario it is concluded that no further detailed studies are needed and that risk assessment can be made and decision can be taken (by applying the DAIMON Decision Support System, DSS).

Example 1: Screening study	Yes	No	Method	Fact Sheet No.
Presence of dumped munition	X		Sonar, ROV, Camera	1.1-1.3
- chemical munitions?		X	Sonar, ROV, Camera, Munitions Catalogue	1.1-1.3
- conventional munitions?	X		Sonar, ROV, Camera, Munitions Catalogue	1.1-1.3
Chemical screening of sediment	X		Chemical screening for selected TNT/-metabolites in sediments	2.3
	-	-	Chemical screening for selected CWA-related compounds in sediments	2.1-2.2
Fish in situ biomarker 1		X	Externally visible fish diseases	3.16
Fish in situ Biomarker 2	X		Liver tumors in fish	3.25
Fish in situ Biomarker 3	X		Micronucleus assay	3.26
RESULTS: Dumped conventional munitions found and TNT–related compounds detected in sediment. Two out of three biomarkers responded and, thus, fish are regarded as affected. Detailed study not required , risk assessment can be done and decisions can be taken.				

Figure 5: Scenario 1, results of the Screening Study

The second scenario describes a different case where the Screening Study did not provide clear results (Figure 6a).

In this case, munitions was found and was identified as chemical munitions, but based on the chemical screening applied there was no indication of the presence of chemical warfare agents in the sediment. Only one of the three biomarkers responded. Based on the overall results of the Screening Study, there was some indication of a problem; however, the results were not regarded as sufficient for a valid assessment. Therefore, a decision was made that an additional Detailed Study is needed to provide more clear evidence. In this Detailed Study (see Figure 6b), some additional methods were applied: Chemical sediment screening was extended to include more CWA-related substances. Furfthermore, a mussel exposure experiment was carried out and CWA was detected in exposed mussels. Two of the three biomarkers measured in exposed mussels responded and the mussels were, thus, regarded as affected by CWA. Taking the results of the Screening and the Detailed Study together, there was evidence of adverse impacts of CWA on biota. Therefore, no further studies are needed, risk assessment can be done and decisions can be taken.

Example 2a: Screening study	Yes	No	Method	Fact Sheet No.
Presence of dumped munition	X		Sonar, ROV, Camera	1.1-1.3
- chemical munitions?	X		Sonar, ROV, Camera, Munitions Catalogue	1.1-1.3
- conventional munitions?		X	Sonar, ROV, Camera, Munitions Catalogue	1.1-1.3
Chemical screening of sediment	-	-	Chemical screening for selected TNT/-metabolites in sediments	2.3
		X	Chemical screening for selected CWA-related compounds in sediments	2.1-2.2
Fish in situ biomarker 1		X	Externally visible fish diseases	3.16
Fish in situ Biomarker 2	X		Liver tumors in fish	3.25
Fish in situ Biomarker 3		X	Micronucleus assay	3.26

RESULTS: Chemical munitions found, but no munitions-related compounds detected in sediment. One out of three biomarkers responded, it is thus not clear if organisms are affected. Therefore, a Detailed Study is required before risk assessment can be done and decisions can be taken.

Figure 6a: Scenario 2, results of the Screening Study

Example 2b: Detailed study	Yes	No	Method	Fact Sheet No.
Presence of dumped munition	-		Sonar, ROV	1.1-1.3
Chemical munitions?	-		Sonar, ROV, Camera, Munitions Catalogue	1.1-1.3
Conventional munitions?		-	Sonar, ROV, Camera, Munitions Catalogue	1.1-1.3
Chemical screening of sediment	X	-	Chemical screening for more/other CWA-related compounds in sediments	2.1.-2.2
Chemical screening biota (exposed mussels)	X		Chemical screening for CWA-related compounds in mussels	2.9
Fish in situ biomarker 1		-	Externally visible fish diseases	3.16
Fish in situ biomarker 2	-		Liver tumors in fish	3.25
Fish in situ biomarker 3		-	Micronucleus assay	3.26
Mussel exposure biomarker 1	X		Lysosomal membrane stability	4.1, 3.18
Mussel exposure biomarker 2		X	AChE inhibition	4.1, 3.24
Mussel exposure biomarker 3	X		Micronucleus assay	4.1, 3.26

RESULTS: CWA-related compounds detected in sediment and in exposed mussels. Two out of three exposure biomarkers responded, and thus mussels are considered affected. Therefore, risk assessment can be done and decisions can be taken.

Figure 6b: Scenario 2, results of the Detailed Study. Measurements marked in red were added to the measurements already made in the Screening Study (see Figure 6a)

8. Conclusions and outlook

It has to be emphasized that the DAIMON EcoTox Toolbox is a pragmatic approach to combine the measurement of indicators and the first steps in the assessment process and in decision making. The design of the Toolbox is based on practical experience made during the DAIMON project and previous munitions-related projects. It is a robust, easy-to-apply and easy-to-communicate approach, but its full strength can only be utilized when all components recommended are addressed (presence of munitions, identification of munitions, target-driven chemical measurements and biomarker measurements). The use of only one of these components would not be sufficient to identify and assess effects of toxic munitions compounds on biota. For instance, the finding of biological effects alone would not be indicative of munitions effects, because such effects can also be caused by other contaminants or, e.g. in case of the more general unspecific fitness/health biomarker, by general stress.

The DAIMON EcoTox Toolbox is considered ready for application and for practical testing. It is evident that modifications are required based on experience made and in order to meet end user requirements. This will be a process and a task to be addressed in the DAIMON 2 project started in 2019.

9. References

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ANNEX 1 – DAIMON EcoTox Toolbox Fact Sheets

(see separate files)