

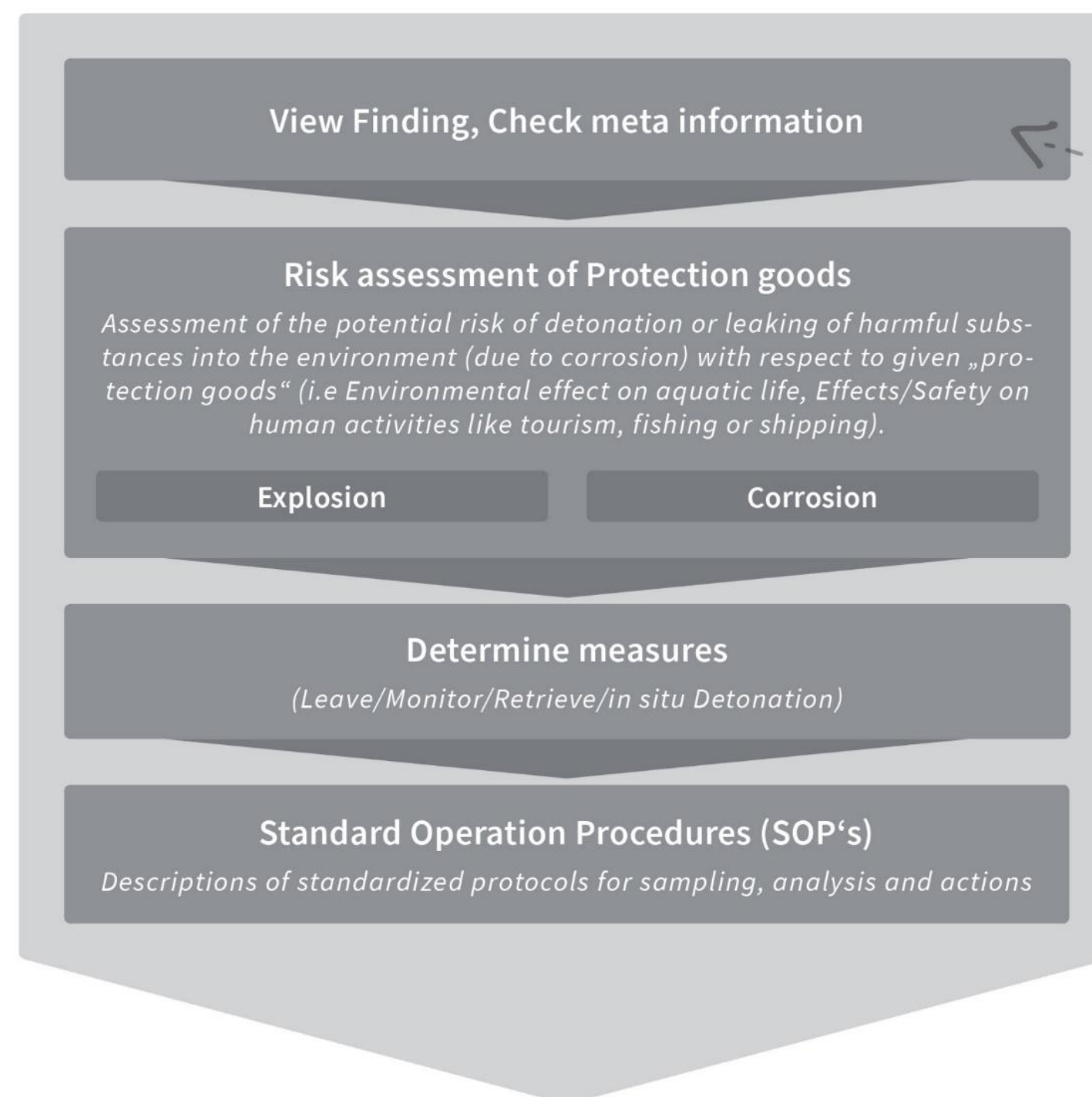
DAIMON DSS-työkalu ja EcoTox Toolbox

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SYKE/merikeskus
15.4.2021

Mereen upotettujen ammusten riskinarviointityökalu päätoksentekijöille



Expert Assessment



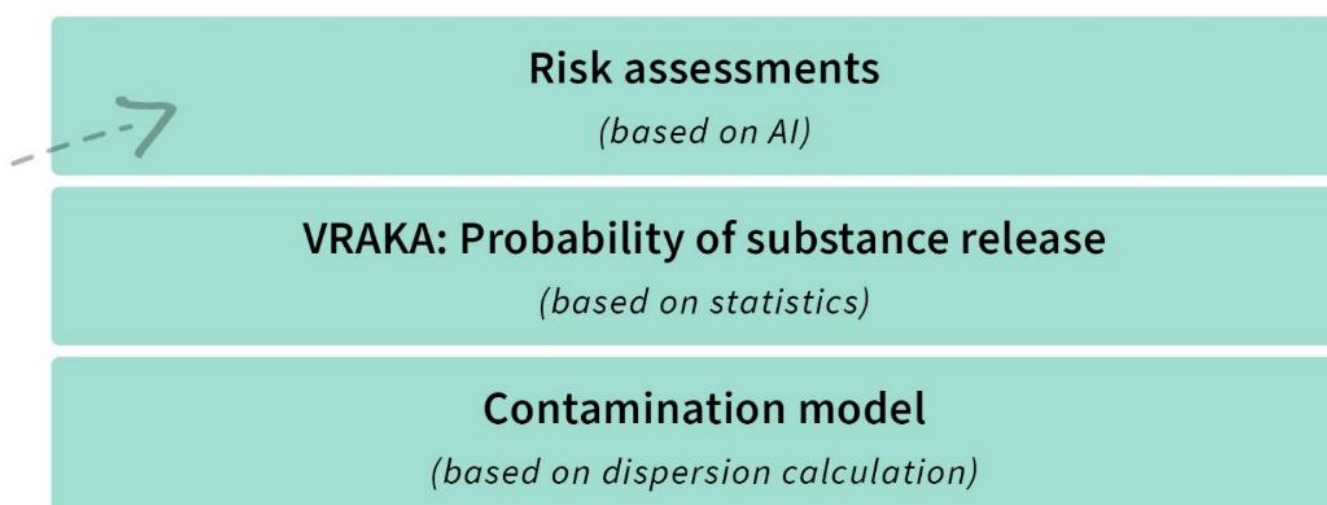
Artificial Intelligence



User

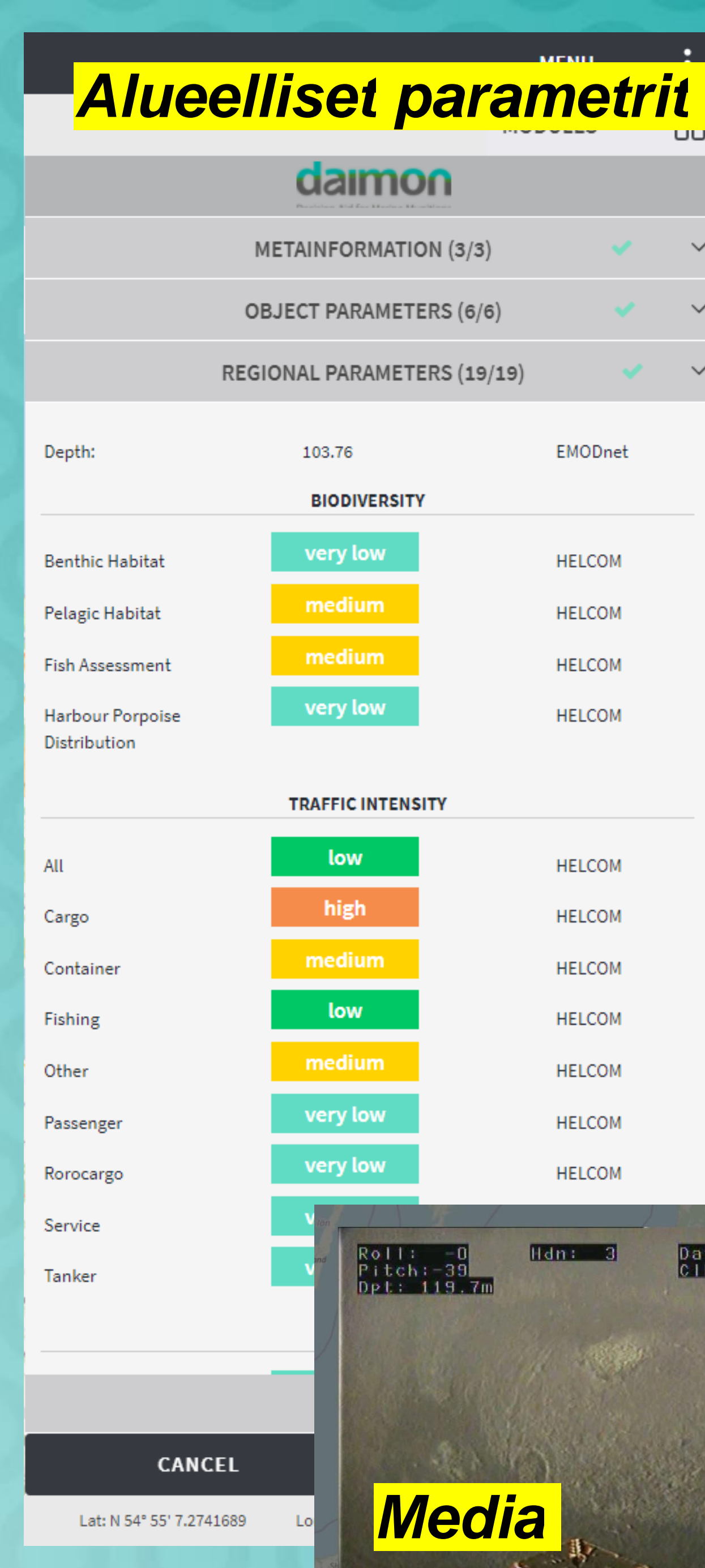
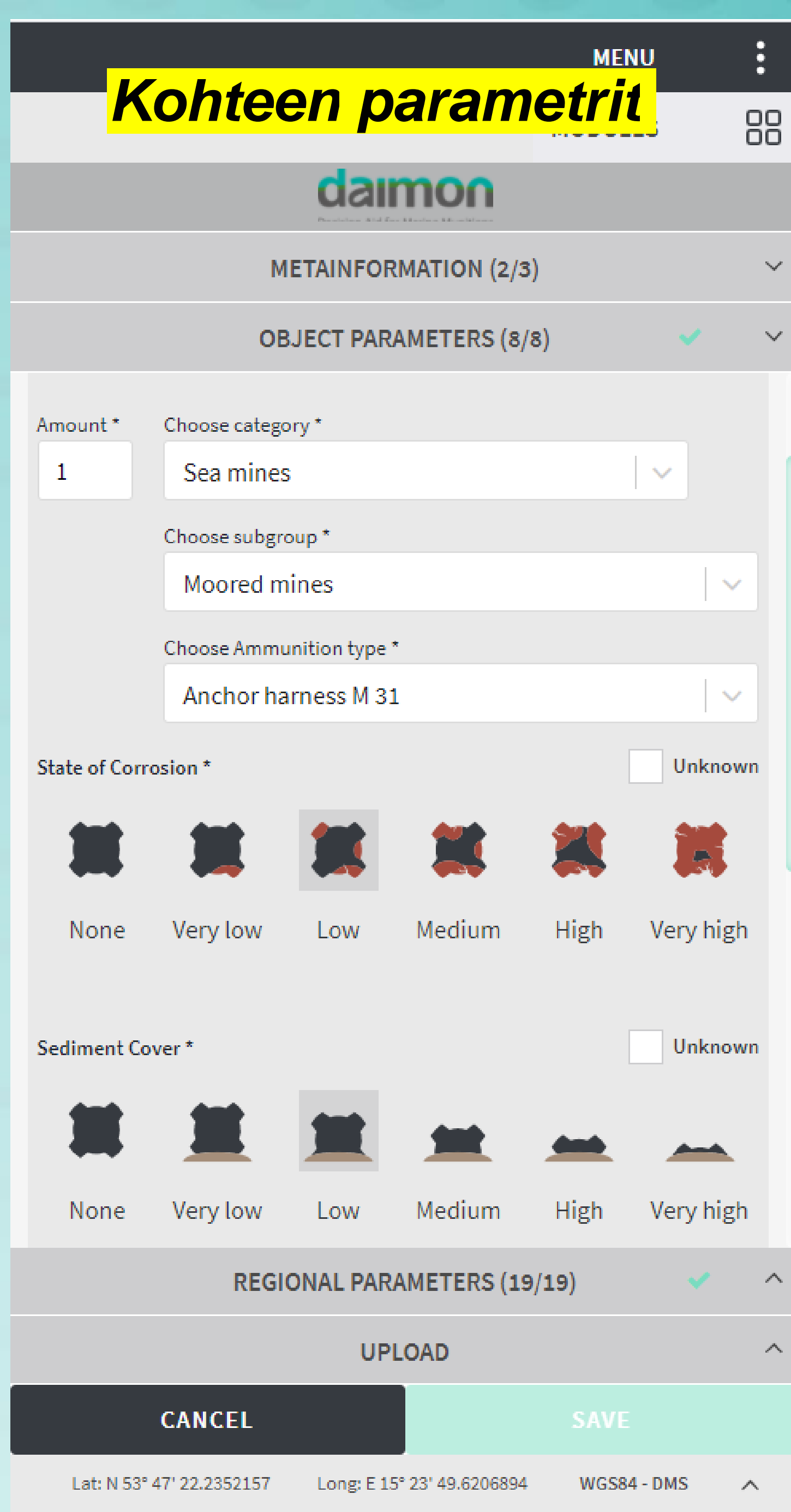
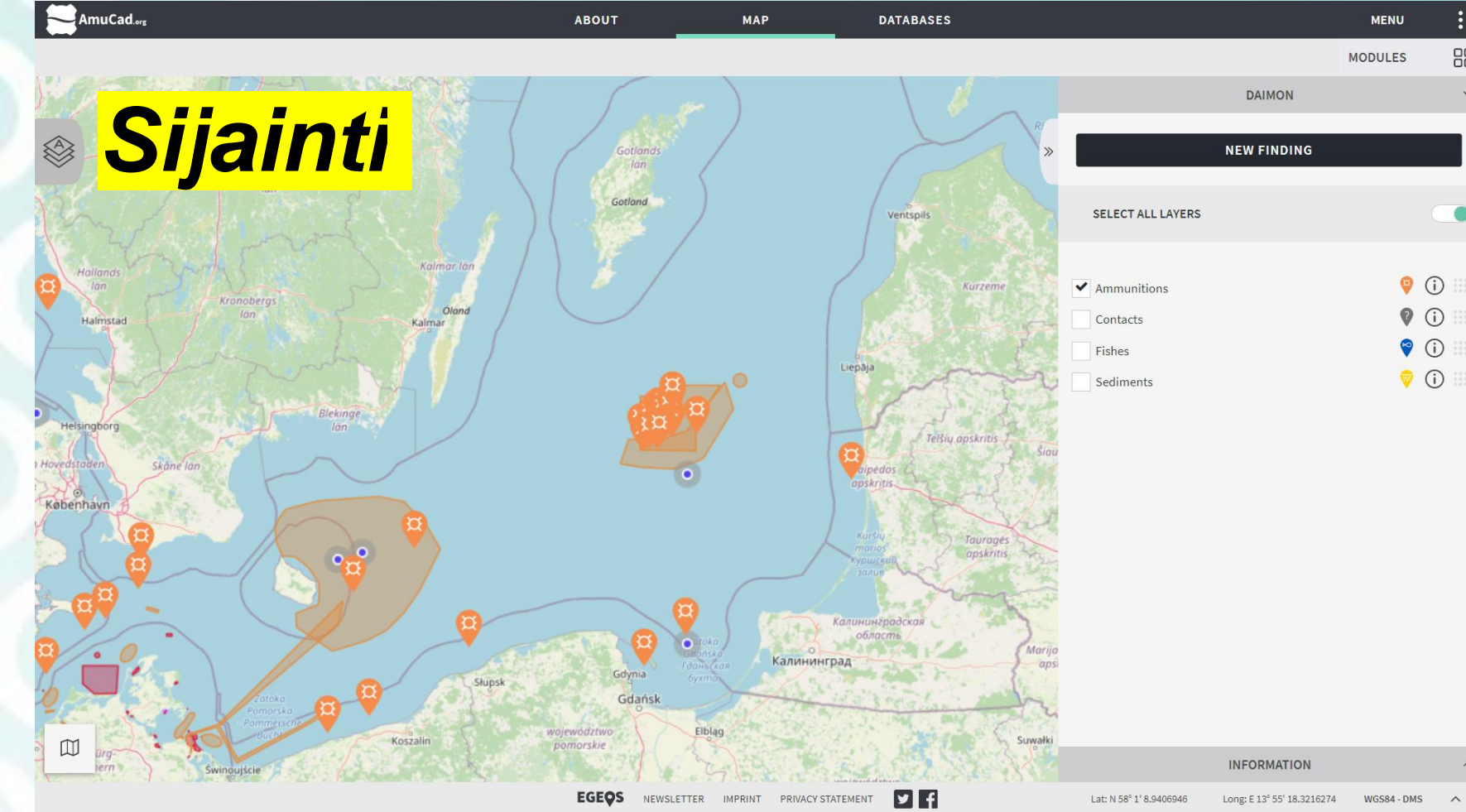


Decision Support



Training

Online Course and Workshops



Karttapohjainen verkkosovellus, joka kokoaa relevanttia informaatiota upotettujen ammusten tilasta ja riskistä



Riskinarviot

- tilastollinen todennäköisyys kohteen rikkoutumiselle ja haitallisten aineiden pääsemiselle mereen
- riski eri tilanteissa ja merellä tapahtuvissa aktiviteeteissa ja tekoaälyyn perustuvat ehdotukset toimenpiteiksi
- ehdotukset toimenpiteiksi kohteen käsittelyn suhteen

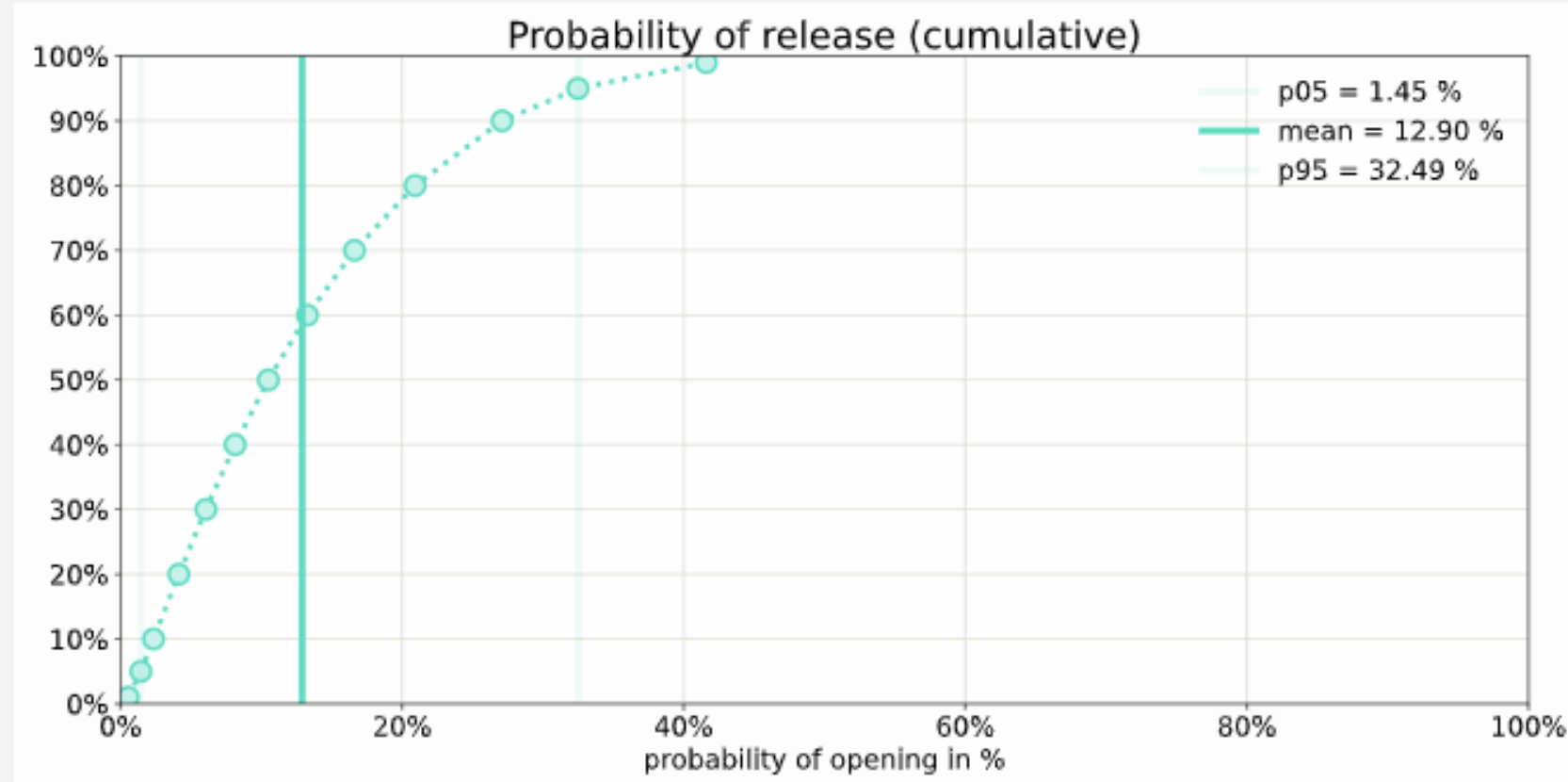


Figure 1: The relative risk value was presented as a cumulative graph. The solid vertical line represents the mean value.

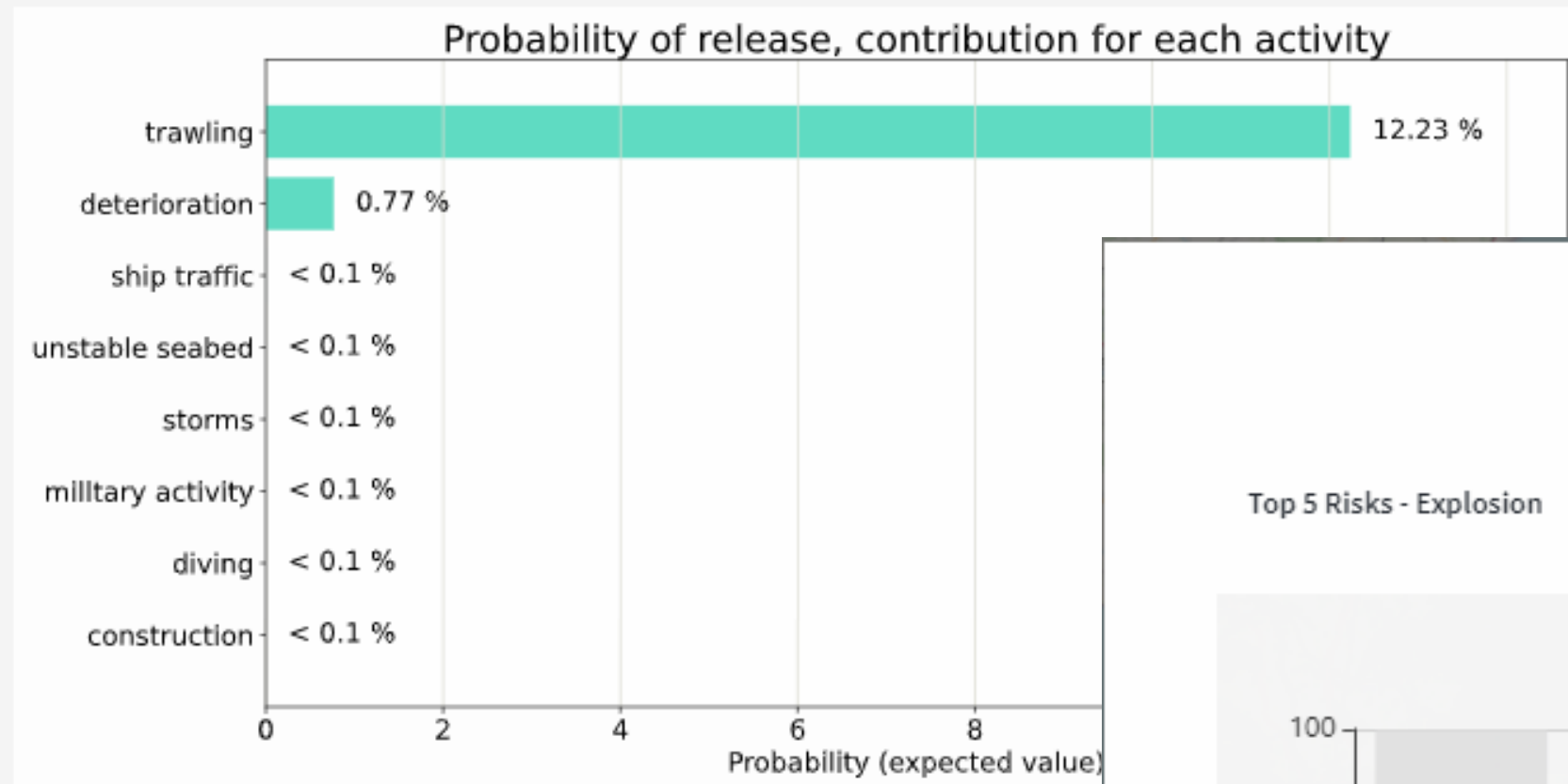


Figure 2: Probability of release (mean value) for each considered activity.



DSS ASSESSMENT

No2777 (Ammunition)

Top 5 Risks - Explosion | **Top 5 Risks - Corrosion**

Risk Category	Value
Flora/Fauna	25
Tourism	25
Shipping	5
Divers	0
Fisheries	0

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The results shown here are suggestions made from datasets that are used for training purposes. Due to the BETA stage of the application, they currently can not be used as an official basis for decision-making. These suggestions are not released for use in real life situation.



Raportti päätöksenteon tueksi

Kerätty data ja niistä DSS:n avulla johdetut suositukset tulostettavassa muodossa tukemaan päätöksentekoa

DECISION SUPPORT REPORT

DECISION SUPPORT REPORT - AMMUNITIONS

User: DaimonTrainer Organisation: Decision Aid for Marine Munitions Date: 2020-10-03 11:44
Coordinates: 19.1439509, 54.8074370

The results shown here are suggestions made from datasets that are used for training purposes. Due to the BETA stage of the application, they currently can not be used as an official basis for decision-making. These suggestions are not released for use in real life situation.

OVERVIEW

Coordinates: 19.1439509, 54.8074370 Date, Time: 2020-10-03 11:44



Description: A single bullet found by ROV

TOP 5 RISKS - EXPLOSION

Protection Goods	Risk	Training-Datasets
Tourism	38 %	6
Flora/-Fauna	29 %	51
Shipping	3 %	35
Divers	0 %	38
Fisheries	0 %	93

TOP 5 RISKS - CORROSION

Protection Goods	Risk	Training-Datasets
Shipping	32 %	3
Tourism	29 %	88
Fisheries	27 %	38
Divers	0 %	24
Flora/-Fauna	0 %	29

DESCRIPTION

The top 5 scaled risks for spontaneous detonation & corrosion per protection good, sorted from high to low. The indicated risks are shown on a relative scale from 0 to 100%. Color coding indicates the different risk classes where very low: 0 - 20%; low: 20-40%; intermediate: 40-60%; high: 60-80% and very high: 80-100%

The EcoTox Toolbox – “from suspicion to decision”

Epäily:

Määrätyllä merialueella on menneisyydessä tapahtunut ammusten upotusta.

Kysymys:

Aiheuttavatko nämä ammuksset ja niiden sisältämät myrkylliset kemikaalit uhkaa alueen merieliöstölle?

The EcoTox Toolbox – “from suspicion to decision”

Kysymykset

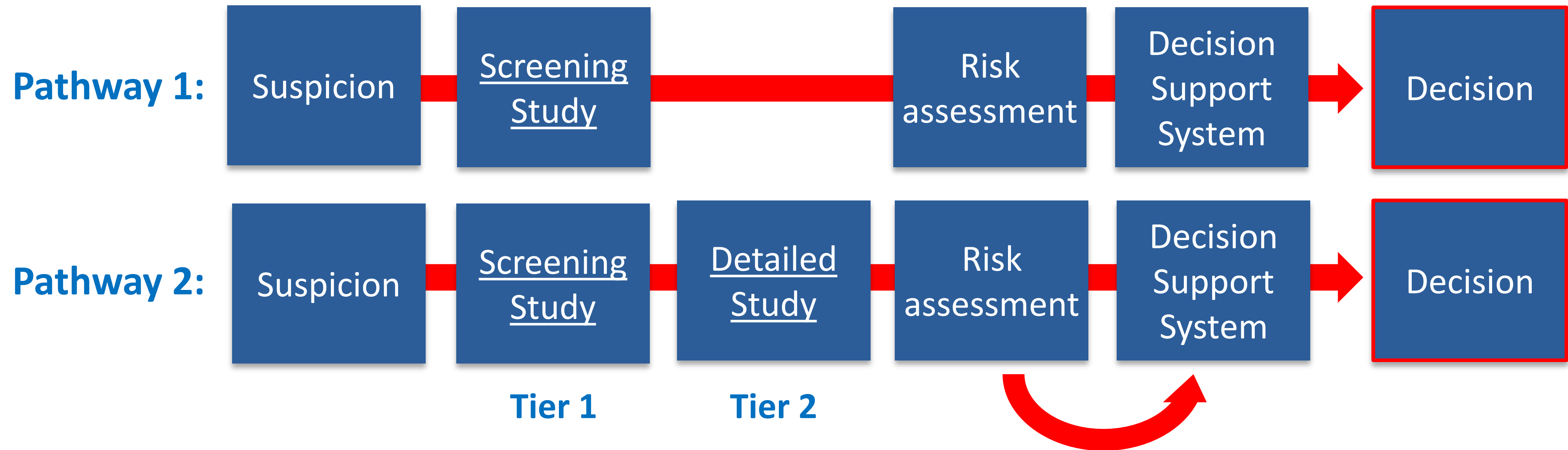
- onko alueella ammuksia?
- vuotaako niistä haitallisia aineita ympäristöön?
- onko vuotavilla aineilla biologisia vaikutuksia?
- onko yleisesti ottaen syytä huoleen?
- mitkä ovat suositeltavat toimenpiteet?

The EcoTox Toolbox – “from suspicion to decision”

Ratkaisu:

- DAIMON EcoTox Toolbox, koska se tarjoaa
 - loogisen konseptin ja strategian
 - soveltuvat menetelmät
 - menelmien kuvaukset (fact sheets)

TOOLBOX PATHWAYS



SUSPICION: Ecological risk due to dumped munitions?

TIER 1: SCREENING STUDY

Check availability of data facilitating a risk assessment

sufficient data for risk assessment available

no or insufficient data for risk assessment

Run risk assessment

Feed data into Decision Support System

Decide on actions to be taken

optical/sonar/
magnetometric
screening

chemical screening
sediment

biological effects
screening

3 Options

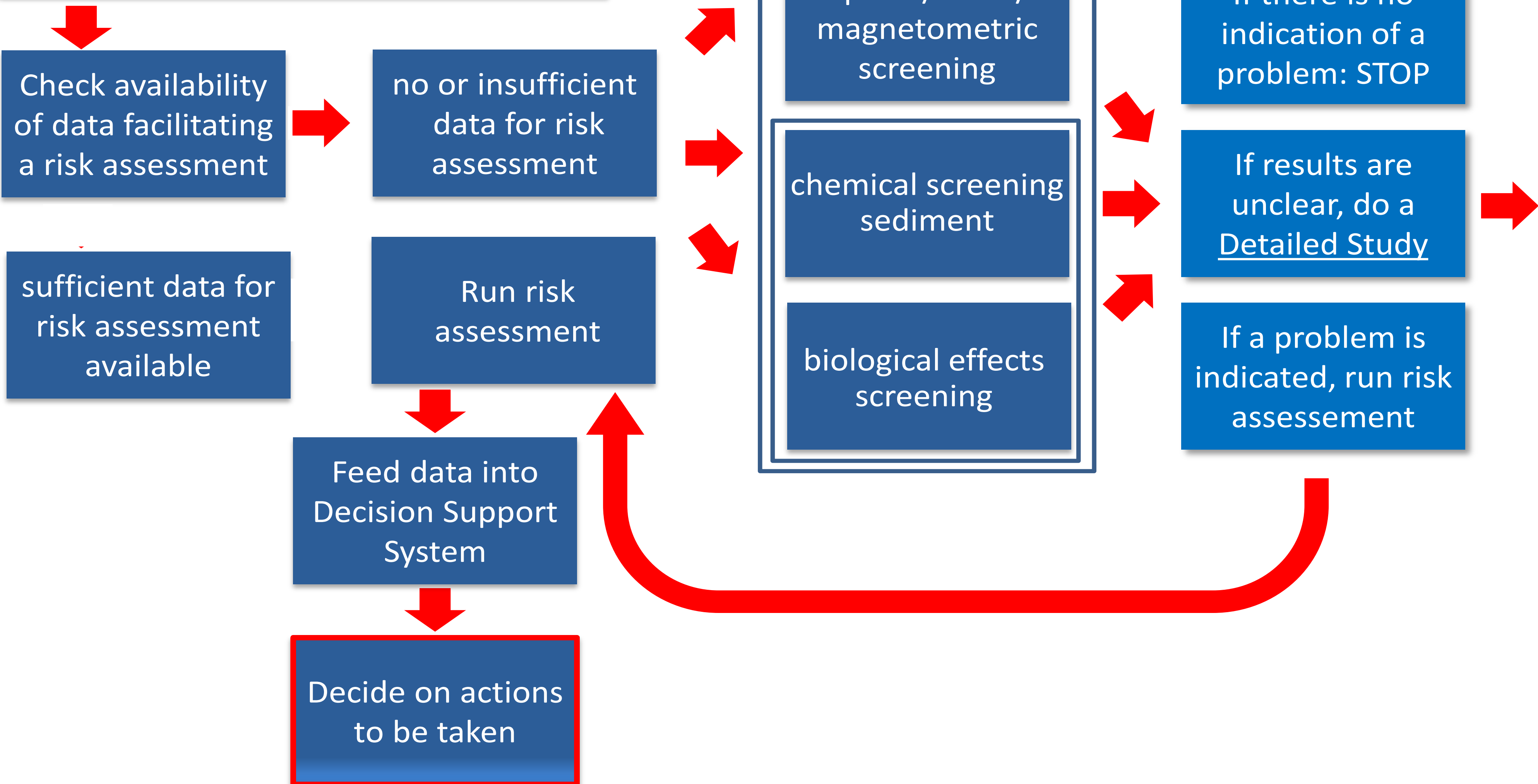
If there is no indication of a problem: STOP

If results are unclear, do a Detailed Study

If a problem is indicated, run risk assessment

SUSPICION: Ecological risk due to dumped munitions?

TIER 1: SCREENING STUDY



SUSPICION: Ecological risk due to dumped munitions?

If the results from the Screening Study were unclear

TIER 2: DETAILED STUDY

Do more chemical measurements

Do more bioeffect measurements

Apply other approaches

2 Options

If there is no indication of a problem: STOP

If a problem is indicated, run risk assessment

Run risk assessment

Feed data into Decision Support System

Decide on actions to be taken

Työkalut



Työkalut



Presence of munitions

Release of hazardous substances

Biological effects *in situ*

Other approaches

Data analysis & assessment

Decision support



Työkalut: ryhmittely

Munitions detection & identification

- Side scan sonar
- Sub-bottom profiler
- Magnetometry
- Neutron Activation Analysis
- Camera systems
- AUV, ROV
- Modelling

Biological effects

- Biomarker battery
- General, specific biomarkers
- Fish, mussel

Other approaches

- *in situ* exposure (Fish, Mussels)
- Lab toxicity tests
- Sediment/water bioassays

Data analysis & assessment

- Statistics
- Assessment criteria
- Integrated risk assessment

Hazardous substances

- Chemical analysis of CWA and degradation products (e.g. GC-MS, LC-HESI /MS/MS)
- Chemical analysis of explosives and degradation products (e.g. LC-QQQ-MS)

Decision support

- Decision Support System

Biologisten parametrien valinta

Indicator Category	Parameter A	Parameter B	Parameter C
1: Fitness	1.1: Fulton's condition factor (CF)	1.2: Hepatosomatic index (HSI)	
2: General stress	2.1: Glucose (blood)	2.2: Oxidative stress (liver)	2.3: Macromolecular defence (liver)
3: Disease/Pathology	3.1: Gross diseases/parasites (external/internal)	3.2: Histopathology (liver/kidney)	3.3: Lysosomal membrane stability (liver/head kidney)
4. Immunosuppression (-toxicity)	4.1: Haematology (Hc, Lc, Hb) (blood)	4.2: Differential white blood cell count (blood)	4.3: Macrophage aggregates (spleen)
5. Neurotoxicity	5.1: Acetylcholinesterase inhibition		
6. Carcinogenicity	6.1: Histopathology (liver)	6.2: Macroscopic neoplasms (liver)	
7. Genotoxicity	7.1: Micronucleus Assay (Blood)	7.2: Comet Assay	7.3: Genomics

Tulosten tulkinta



Assessment Criteria

EAC:
environmental
assessment
criteria

BAC:
background
assessment
criteria

Example: Biomarkers in fish

Fish No.	Biomarker	Response	BAC	EAC
1	A	0.53	<4.0	>20.0
	B	178.31	<100.0	>250.0
	C	Tumour: No	No	Yes
2	A	4.78	<4.0	>20.0
	B	99.87	<100.0	>250.0
	C	Tumour: Yes	No	Yes
3	A	3.99	<4.0	>20.0
	B	543.22	<100.0	>250.0
	C	Tumour: Yes	No	Yes

"Fact Sheets"



Decision Aid for Marine Munitions



DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota



Assessment category: Other approaches

Toolbox component: Lab toxicity studies

Fact Sheet x.x: Comet Assay (applied to zebrafish embryos)

Author: Daniel Koske, Thünen Institute of Fisheries Ecology

What is it?

Ecotoxicological test system to quantify genotoxicity in single cells after exposure to chemicals. The Comet Assay can be performed with different cell types, cell cultures or whole fish embryos and detects single strand breaks and double strand breaks under alkaline conditions. The test can be used both *in vivo* and *in vitro*.

What does it tell you?

The Comet Assay applied to zebrafish (*Danio rerio*) embryos is used to quantify DNA damage in newly fertilized zebrafish eggs caused by a specific chemical. In comparison with the control, the genotoxic risk of a chemical can thus be determined. Effects of chemicals on DNA that may lead to genotoxicity can cause severe damage such as mutations or carcinogenesis. In addition, genotoxicity can ultimately affect individual fitness and populations if it leads to reproductive stress or altered genotypic diversity (Anderson et al. 1994; Hylland et al. 2017). Heavily damaged DNA migrates further in the electric field than less damaged DNA, resulting in a fluorescent 'comet' behind the nucleus.

Type of Indicator (tick box)

- non-specific stress indicator
- specific for groups of contaminants incl. CWA or explosives
- CWA-specific indicator
- specific for substances related to explosives (e.g. TNT)

How to measure it?

According to Fact Sheet No. XX zebrafish eggs are exposed to the test substance for 48 hours. The test concentrations should be chosen so that they are below the acute toxic threshold. Test solutions should be renewed after 24 hours and eggs should be checked for sublethal effects.

After 48 hour of exposure zebrafish embryos are used for the Comet Assay under alkaline conditions (Singh et al. 1988) according to the procedure described by Kosmehl et al. (2008).

Matrix: Whole zebrafish embryos.

Main Equipment: Electrophoresis unit including power supply, troughs for horizontal and vertical storage of the slides, heating block or microwave, refrigerated centrifuge for reaction tubes, vortex mixer, ice machine, fluorescence microscope (100x/200x magnification) with possibility of excitation at 490 nm and emission at 530 nm, microscope camera, hemocytometer, reaction tubes, frosted microscope slides and cover glasses, 70 µm filter mesh and 2 ml syringes, pipettes, various glassware including 1 l bottles.

Sample size: To determine the genotoxicity of a chemical, the Comet Assay should be performed in triplicate with twenty zebrafish embryos per replicate to ensure sufficient amount of DNA in the sample.

Additional notes:

If necessary, the exposure time of embryos can be prolonged up to 120 hours after fertilization as they are not subject to animal testing regulations until then.

Filtering the homogenized embryos is important to ensure that single cells are present in the samples for later analysis.

Midori Green (Biozym) can be used as an alternative to ethidium bromide as DNA fluorescent dye.

How to analyse and assess the data?

Image analysis of the comets to determine DNA damage can be performed with the open source program CASP (Końca et al. 2003), proprietary software solution are also available. Several parameters to quantify the DNA migration are given by the software and can be used for evaluation. Usually, at least 50 comets per gel should be analyzed (Møller and Loft 2014). For instance, the percentage of DNA in the tail of each comet is plotted and used for statistical analysis. In order to assess the genotoxicity of a given chemical, the control is used as a reference for statistical comparisons. Parametric as well as non-parametric can be applied to the Comet Assay data and the experimental design determines the type of statistical analysis (Møller and Loft 2014).

References

Esimerkkejä



Example 1: Screening study	Yes	No	Method	Fact Sheet No.
Presence of dumped munition	X		Sonar, ROV, Camera	XXX
- chemical munitions?		X	Sonar, ROV, Camera, Catalogue	XXX
- conventional munitions?	X		Sonar, ROV, Camera, Catalogue	XXX
Chemical screening of sediment	X		Chemical screening for TNT /- metabolites in sediments	XXX
Fish biomarker 1		X	3.1: Externally visible fish diseases	XXX
Fish Biomarker 2	X		6.2: Liver tumors in fish	XXX
Fish Biomarker 3	X		7.1: Micronucleus assay	XXX

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.

Esimerkkejä



Example 2a: Screening study	Yes	No	Method	Fact Sheet No.
Presence of dumped munition	X		Sonar, ROV, Camera	XXX
- chemical munitions?	X		Sonar, ROV, Camera, Catalogue	XXX
- conventional munitions?		X	Sonar, ROV, Camera, Catalogue	XXX
Chemical screening of sediment		X	Chemical screening for CWA-related compounds in sediments	XXX
Fish biomarker 1		X	Externally visible fish diseases	XXX
Fish Biomarker 2	X		Liver tumors in fish	XXX
Fish Biomarker 3		X	Micronucleus assay	XXX

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.

Esimerkkejä



<u>Example 2b: Detailed study</u>	Yes	No	Method	Fact Sheet No.
Presence of dumped munition	-		Sonar, ROV	XXX
Chemical munitions?	-		Sonar, ROV, Camera	
Conventional munitions?		-	Sonar, ROV, Camera	
Chemical screening of sediment	X	-	Chemical screening for <u>more/other</u> CWA-related compounds in sediments	XXX
Chemical screening biota (exposed mussels)	X		Chemical screening for CWA-related compounds in mussels	XXX
Fish in situ biomarker 1		-	Externally visible fish diseases	XXX
Fish in situ biomarker 2	-		Liver tumors in fish	XXX
Fish in situ biomarker 3		-	Micronucleus assay	XXX
Mussel <u>exposure</u> biomarker 1	X		Lysosomal membrane stability	XXX
Mussel <u>exposure</u> biomarker 2		X	AChE inhibition	XXX
Mussel <u>exposure</u> biomarker 3	X		Micronucleus assay	XXX

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.



Kiitos!

