



**DAIMON2**  
**ECO TOX Toolbox**  
Procedures & Approach

*On-line project meeting, 15th June 2021*

# ECOTOX Toolbox

How it works?

Tools in the box

Three questions, one answer

# Tools in the box

Detection and identification

Sonar; AUV; ROV

Sampling

Gravity Corer; ROV; Box & Van Veen

Safety

Sampling; People

Chemical analysis

CWA; Explosives – biota & sediment

Biota impact

Biomarkers, accumulation, toxicity

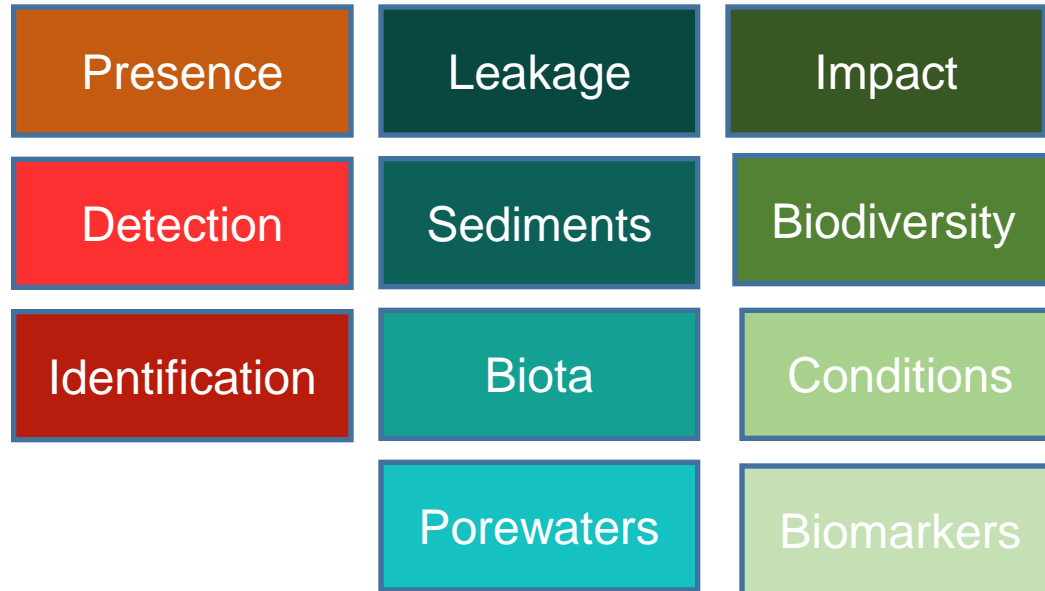
Habitat condition

Oceanography, water & sediment chemistry

Data analysis

Risk Matrix

# 3 Questions



## DAIMON Ecotox Toolbox results (examples)

Question 1 **Yes** Question 2 **Yes** Question 3 **Yes**

Munition is present in the area, substances are leaking, contamination in organisms/sediment and biological effects have been detected. Munition may affect the ecosystem here.

Question 1 **Yes** Question 2 **Yes** Question 3 **No**

Munition is present in the area, substances are leaking, contamination in organisms/sediment but no biological effects are present. Munition is present but not likely to affect the ecosystem here. Situation is unclear and should be further observed or investigated with Toolbox in advanced mode or with DSS.

Question 1 **Yes** Question 2 **No** Question 3 **No**

Munition is present in the area, substances are not leaking to sediment/organisms and no biological effects are present. Munition is not likely to affect the ecosystem here.

Question 1 **No** Question 2 **No** Question 3 **No**

Munition is not present and no contamination or effect have been observed. No action is needed.

Question 1 **No** Question 2 **Yes** Question 3 **Yes**

No munition is present but chemicals have been detected in organisms/sediment and biological effects are present. In this situation, the cause of the contamination should be looked up – maybe with DSS. Then Toolbox analysis should be repeated.

# DAIMON A 2.5 Toolbox

## The tools: grouping

### 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

### Hazardous substances

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

### Data analysis & assessment

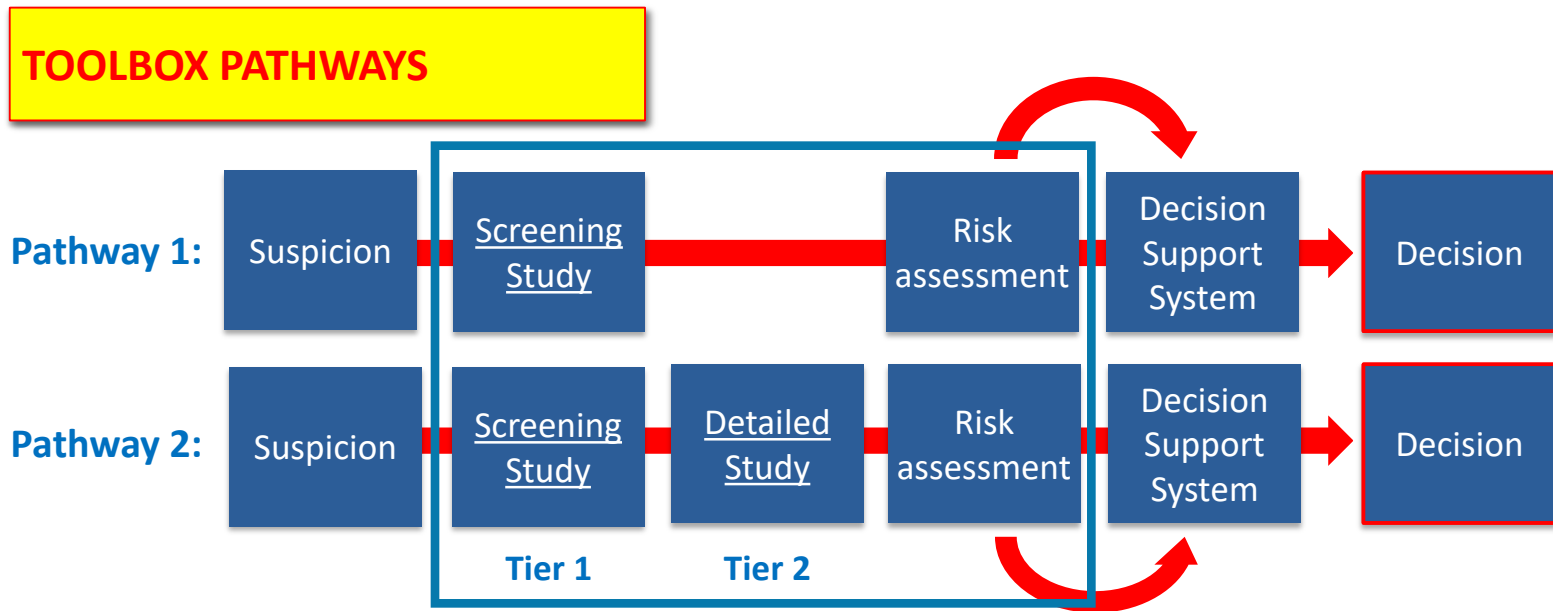
- Statistics
- Assessment criteria
- Integrated risk assessment

### Decision support

- Decision Support System

# ECOTOX Toolbox

## Concept & strategy

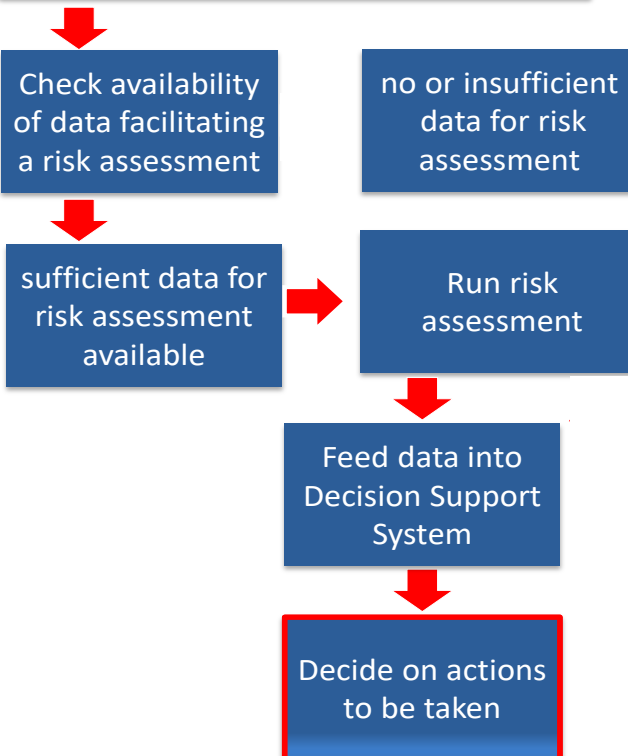


# ECOTOX Toolbox

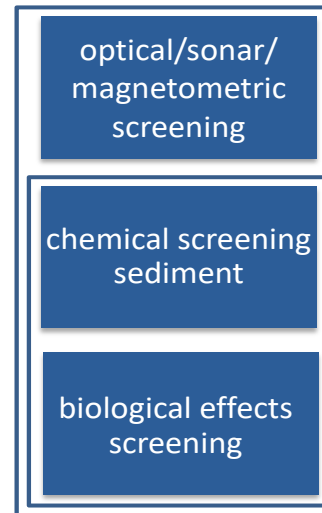
## Tier 1: Screening Study

**SUSPICION: Ecological threat due to dumped munitions?**

### **TIER 1: SCREENING STUDY**



### Screening



### 3 Options

If there is no indication of a problem: STOP

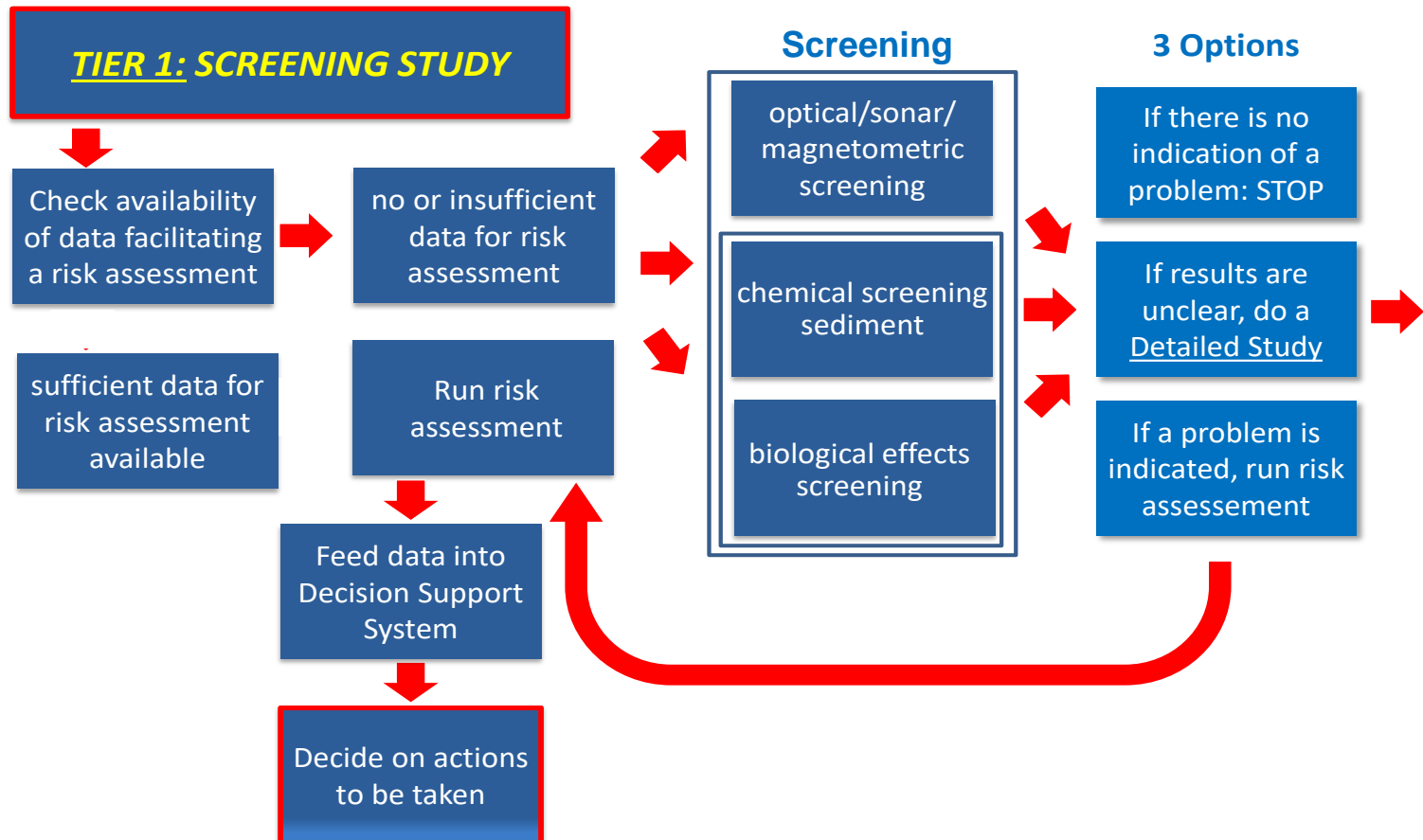
If results are unclear, do a Detailed Study

If a problem is indicated, run risk assessment

# ECOTOX Toolbox

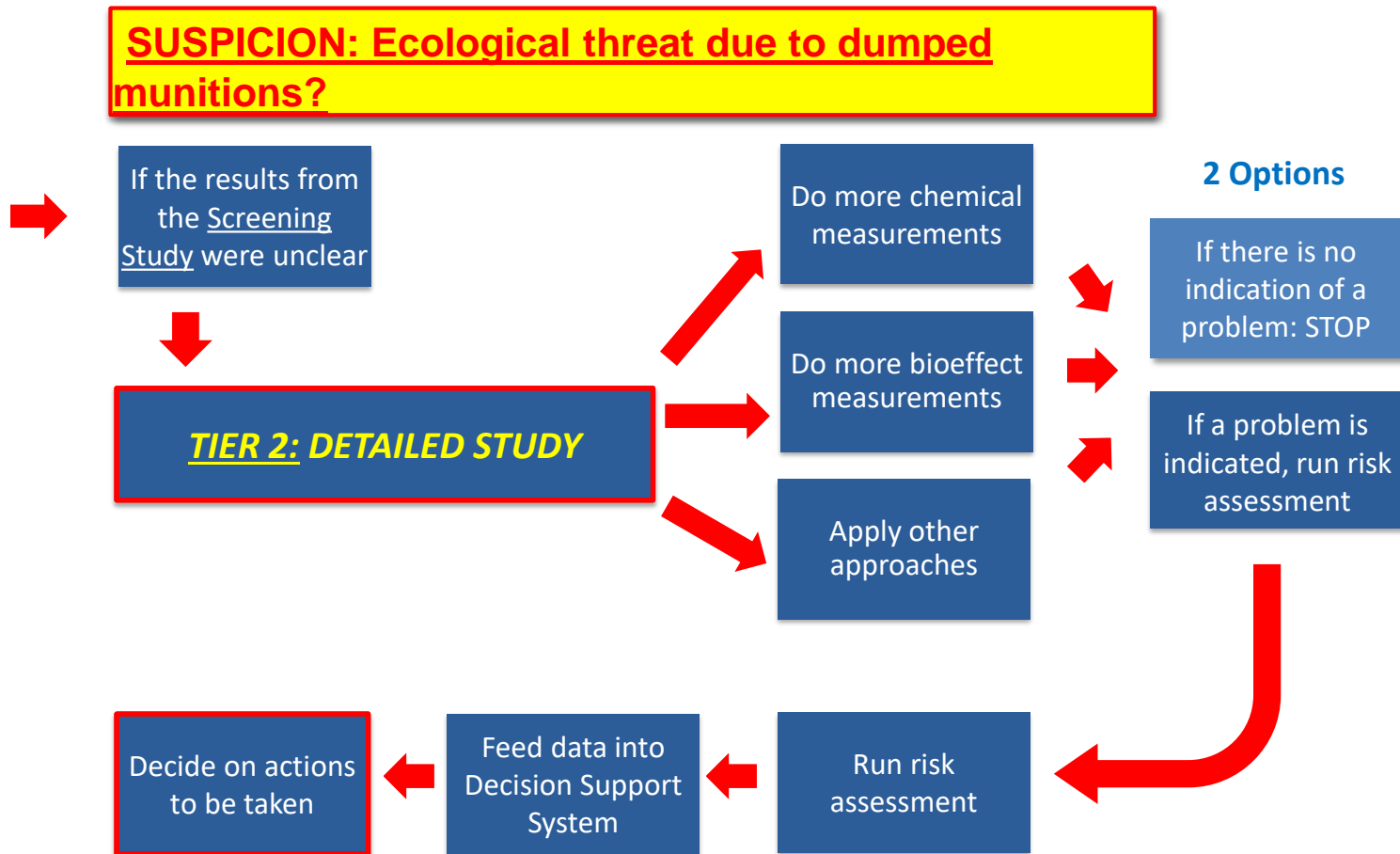
## Tier 1: Screening Study

**SUSPICION: Ecological threat due to dumped munitions?**



# ECOTOXToolbox

## Tier 2: Detailed Study



## DAIMON A 2.5 Toolbox: How to select tools (e.g., *in situ* fish biomarkers)

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

## DAIMON A 2.5 Toolbox: How to select tools (e.g., fish biomarkers)

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

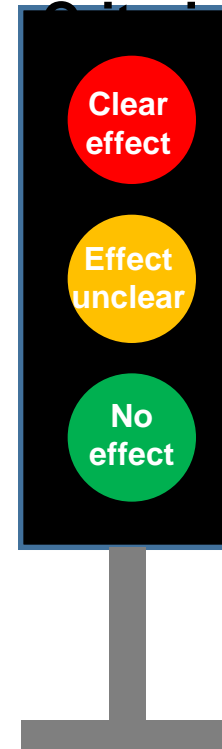
**Indicator relevance:** (1) non-specific stress indicator  
 (2) specific for groups of contaminants incl. CWA or explosives  
 (3) CWA-specific indicator  
 (4) specific for substances related to explosives (e.g.,

# ECOTOX Toolbox: How to select tools and how to interpret results

## Example: *in situ* Biomarkers Fish

- Take samples and measure biological response
- Select 3 biomarkers based on questions to be answered
- Compare results with Assessment Criteria (BAC/EAC)
- Do assessment by biomarker based on Assessment Criteria

### Assessment



**EAC:** environmental assessment criteria

**BAC:** background assessment criteria

# ECOTOX Toolbox

## How to apply the Toolbox (examples)

<b>Example 1: Screening study</b>	<b>Ye s</b>	<b>No</b>	<b>Method</b>	<b>Fact Sheet No.</b>
<b>Presence of dumped munition</b>			Sonar, ROV, Camera	XXX
- chemical munitions?			Sonar, ROV, Camera, Munitions Catalogue	XXX
- conventional munitions?			Sonar, ROV, Camera, Munitions Catalogue	XXX
<b>Chemical screening of sediment</b>			Chemical screening for selected TNT/-metabolites in sediments	XXX
			Chemical screening for selected CWA-related compounds in sediments	XXX
<b>Fish in situ biomarker 1</b>			3.1: Externally visible fish diseases	XXX
<b>Fish in situ Biomarker 2</b>			6.2: Liver tumors in fish	XXX
<b>Fish in situ Biomarker 3</b>			7.1: Micronucleus assay	XXX

**RESULTS:**

# ECOTOX Toolbox

## How to apply the Toolbox (examples)

<u>Example 1: Screening study</u>	Ye s	No	Method	Fact Sheet No.
<b>Presence of dumped munition</b>	X		Sonar, ROV, Camera	XXX
- chemical munitions?		X	Sonar, ROV, Camera, Munitions Catalogue	XXX
- conventional munitions?	X		Sonar, ROV, Camera, Munitions Catalogue	XXX
<b>Chemical screening of sediment</b>	X		Chemical screening for selected TNT/-metabolites in sediments	XXX
	-	-	Chemical screening for selected CWA-related compounds in sediments	XXX
<b>Fish in situ biomarker 1</b>		X	3.1: Externally visible fish diseases	XXX
<b>Fish in situ Biomarker 2</b>	X		6.2: Liver tumors in fish	XXX
<b>Fish in situ Biomarker 3</b>	X		7.1: Micronucleus assay	XXX

**RESULTS:**

# ECOTOX Toolbox

## How to apply the Toolbox (examples)

<u>Example 1: Screening study</u>	Ye s	No	Method	Fact Sheet No.
<b>Presence of dumped munition</b>	X		Sonar, ROV, Camera	XXX
- chemical munitions?		X	Sonar, ROV, Camera, Munitions Catalogue	XXX
- conventional munitions?	X		Sonar, ROV, Camera, Munitions Catalogue	XXX
<b>Chemical screening of sediment</b>	X		Chemical screening for selected TNT/-metabolites in sediments	XXX
	-	-	Chemical screening for selected CWA-related compounds in sediments	XXX
<b>Fish in situ biomarker 1</b>		X	3.1: Externally visible fish diseases	XXX
<b>Fish in situ Biomarker 2</b>	X		6.2: Liver tumors in fish	XXX
<b>Fish in situ Biomarker 3</b>	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.

# ECOTOX Toolbox

Methods transferred to  
procedures

Automatic interpretation by DSS

Strategy assisted by DSS

One Integrated tool – from  
environment to management

# daimon

Decision Aid for Marine Munitions  
PRACTICAL APPLICATION

[www.daimonproject.com](http://www.daimonproject.com)

 **Interreg**  
Baltic Sea Region



EUROPEAN UNION

EUROPEAN  
REGIONAL  
DEVELOPMENT  
FUND

