Bioeffects, impact assessment and toolbox

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The Issue

Are dumped war materials causing harm to the Baltic Sea ecosystem?

- munitions containing chemical warfare agents (CWA)
- conventional munitions containing toxic explosives (e.g., TNT, RDX, HMX)
- **toxicity** (effect thresholds)
- exposure → biological effects on local biota
- evidence of exposure
  - bioaccumulation (parent compounds, metabolites/degradation products)
  - effects at different biological levels
    - molecular/biochemical
    - cellular
    - tissue (pathology)
    - physiology
    - reproduction
    - behaviour
Toxicity threshold studies

Toxicity of CWAs: oxidized form and metabolite of Clark I/II

- *in vitro* studies with fish hepatocytes showed that DPA[ox] forms a glutathione conjugate (DPA-SG)
- the main metabolite two orders of magnitude toxic than the DPA[ox] itself
- other novel metabolites of DPA[ox] were also identified using high resolution mass spectrometry

![Graph showing toxicity threshold studies](image)

DPA-SG, glutathione conjugate of DPA[ox]
Toxicity threshold studies

Toxicity of CWAs: Clark I and thiodiglycol (TDG)

- 21-day reproduction test with the water flea (*Daphnia*)
- **Clark I** toxic at environmentally realistic concentrations (NOEC 625 ng l⁻¹)
- **TDG (mustard gas hydrolysation product)** toxic threshold very high (250-500 mg l⁻¹)
Bioaccumulation

CWAs in biota

- sampling in Bornholm, Måseskär and Skagerrak dumpsites (175 individuals in total)
- trace amounts of phenylarsenic CWAs were detected from marine biota samples for the first time
  - 25% of analysed muscle tissue samples contained CWAs

- chemicals were analysed as their oxidation forms
  → total CWAs concentrations in marine biota remain still unknown
Bioeffects

Laboratory exposure of mussels to TNT

Acute (96 h) and chronic (21 d) laboratory toxicity tests
Bioeffects

Laboratory exposure of mussels to TNT

21-day experiment

**Shell closure**
(behavioural response)

Contact: Matthias.Brenner@awi.de
Bioeffects
Laboratory exposure of mussels to TNT

21 day exposure

Lipofuscin accumulation
(metabolic end product of peroxidation processes)

Neutral lipid accumulation
connected to exposure to organic pollutants

Contact: Matthias.Brenner@awi.de
Bioeffects
Field studies: field-collected and transplanted mussels in target areas

- WWII wreck “M451” in the Gulf of Finland
- Finnish Navy divers collected mussels growing on the wreck and on top of the depth charges before the deactivation operation
- reference samples from a nearby coastal area
Bioeffects

Field studies: field-collected and transplanted mussels in target areas

Lysosomal membrane stability (cytotoxicity, general stress)

<table>
<thead>
<tr>
<th></th>
<th>Porkkala reference</th>
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<th>M451 cracked depth charge</th>
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<tbody>
<tr>
<td>Destabilisation time min</td>
<td>a</td>
<td>b</td>
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Lipofuscin accumulation (metabolic end product of peroxidation processes)

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Bioeffects

Field studies: field-collected and transplanted mussels in target areas
Bioeffects
Field studies: field-collected and transplanted mussels in target areas

Mussel caging experiment
• a large (ca. 350 kg) sea mine in the Gulf of Finland
• one cage close to the mine (20 m) and two reference cages 1 and 2 nm from the “hot spot”
Bioeffects
Field studies: hagfish in Skagerrak near a CWA dumpsite
Bioeffects
Field studies: hagfish in Skagerrak near a CWA dumpsite

Foci of cellular alteration in liver tissue (pre-cancer state)
Bioeffects
Field studies: flatfish in Kolberger Heide sea mine dumpsite
Bioeffects
Field studies: flatfish in Kolberger Heide sea mine dumpsite

Metabolism of explosives in-vitro

Genotoxicity of explosives and metabolites

Detection of metabolites in wild fish

Identification of malignant liver tumors in wild fish

Laboratory

Field

Decision Aid for Marine Munitions
The Toolbox
From suspicion to decision

The case
• For a given geographical maritime area there is suspicion that dumping of munitions took place in the past.

The concern
• Do these munitions and their toxic chemical components pose a threat to marine organisms in their habitat?
The Toolbox
From suspicion to decision

The questions

• munitions present?
• hazardous substances released to the environment?
• biological effects?
• overall, a reason of concern?
• actions recommended?
The Toolbox
From suspicion to decision

The solution
• apply the DAIMON Toolbox because it provides
  ▪ concept & strategy
  ▪ selection of appropriate methods
  ▪ method description (fact sheets)
The Toolbox
From suspicion to decision
The Toolbox
From suspicion to decision

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
- Run risk assessment
- Feed data into Decision Support System
- Decide on actions to be taken

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

optical/sonar/magnetometric screening
chemical screening sediment
biological effects screening
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

options:

- 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

Feed data into Decision Support System

Decide on actions to be taken

The Toolbox
From suspicion to decision
**The Toolbox**
From suspicion to decision

**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

Decide on actions to be taken

Feed data into Decision Support System

Run risk assessment
The Toolbox
From suspicion to decision
The Toolbox
From suspicion to decision

**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

**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)

**Data analysis & assessment**
- Statistics
- Assessment criteria
- Integrated risk assessment

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

**Decision support**
- Decision Support System

**Daimon**
Decision Aid for Marine Munitions
Let's get started and fix this damn problem!
Thank you for your attention!