BIOLOGICAL EFFECTS OF WWII CHEMICAL WARFARE AGENTS (CWA) MEASURED IN MUSSELS (MYTILUS TROSSULUS) CAGED AT THE MAJOR DUMPING SITE IN THE BALTIC SEA

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Outline

• Bornholm deep – major CWA dumping site in the Baltic Sea
  – Background
  – Area
  – Environmental risk

• Mussel caging experiment
  – Caging procedure & sites
  – Analyzed biomarkers
  – Results

• Conclusions & Future activities
Background – CWAs in the Baltic Sea

- Ca. 50 000 t of chemical warfare agents (CWAs) were dumped into the Baltic Sea after WWII.


- **CHEMSEA** (Chemical Munitions Search and Assessment)
  - EU Baltic Sea Region Programme (2011–2014)
  - POLAND, FINLAND, GERMANY, SWEDEN, LITHUANIA
  - [www.chemsea.eu](http://www.chemsea.eu)

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<table>
<thead>
<tr>
<th>CWA chemical</th>
<th>Tons</th>
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</thead>
<tbody>
<tr>
<td>Mustard gas</td>
<td>25,000</td>
</tr>
<tr>
<td>Arsenic oil</td>
<td>7,500</td>
</tr>
<tr>
<td>Chloroacetophenone</td>
<td>7,100</td>
</tr>
<tr>
<td>Clark I</td>
<td>1,500</td>
</tr>
<tr>
<td>Clark II</td>
<td>100</td>
</tr>
<tr>
<td>Adamsite</td>
<td>3,900</td>
</tr>
<tr>
<td>Phosgene</td>
<td>5,900</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>2,000</td>
</tr>
<tr>
<td>Tabun</td>
<td>12,000</td>
</tr>
<tr>
<td>Lewisite</td>
<td>Not known</td>
</tr>
</tbody>
</table>
Background – CWAs in the Baltic Sea

- Dumping sites at Bornholm deep and Gotland deep (max. depth 100m).

- Over 500,000 different warfare objects found from the Bornholm dumping site (incl. several wrecks).
  - Corroded & buried into the sediment

- CWA ammunitions (eg. air bombs and mines), shells and containers have been found in large areas outside the “official” dumping sites.
Mustard gas
7 000 tons

Adamsite
1 500 tons

Clark I
~1 000 tons

Triphenylarsine
~100 tons

α-Chloroacetophenone (CN)
500 tons
Mustard gas degradation

Lump of mustard gas

Sanderson & Fauser, DMU-75-00061B, University of Aarhus, 2008.
Environmental risk

Stress responses detected in the laboratory

Blue mussel samples

Fish samples (Baltic cod)

Bioavailable CWAs in biota

CWAs in water

CWAs in sediment
Mussel caging experiment

- May - August 2012
- Collection site of the mussels: "start" and "End"
- Caging: 3 stations, 2 depths (35m ja 65m)
- 400 mussels per cage
- Temperature, salinity and oxygen sensors attached to the cages.
Mussel caging experiment

- Low mortality in all cages.
- Good oxygen conditions at 65m (6.3-4.9 mL/L).
- Cages were also equipped with POCIS passive samplers.
- Samples for all biomarker and chemical analysis as planned.

Surface: Temp 18°C, Sal 7.5 ppm

35m: Temp 10°C, Sal 7.5 ppm

65m: Temp 4°C, Sal 11 ppm
Measured biomarkers

- **Genotoxic & cytotoxicity**
  - Micronuclei and other nuclear abnormalities

- **Neurotoxicity**
  - AChE

- **General stress**
  - Antioxidant enzyme activities
  - Biotransformation
  - Lysosomal membrane stability (Neutral Red retention)
  - Histological markers
  - Bioenergetics (CEA) and condition index
Results

- Cellular energy allocation and condition index
  - Station hot1_65m: available energy, but low condition index.
  - Food conditions at 35m.
• **Histochemical parameters**
  – Lysosomal membrane stability
  – Lipofuscin is an end metabolic product of peroxidation processes.
  – Neutral lipid is indicator of lipidosis induced by toxic organic chemicals.
Results

• AChE – no significant differences!

• GST, CAT, GR activities (65m cages).
Results

• Genotoxic and cytotoxic responses
  – Fragmented & apoptotic cells at hotspot sites.
Conclusions & Future activities

• Highest biomarker responses at all studied biological levels were detected at 65m hotspot sites (especially hot1_65m).

• Results from chemical analysis (sediments and mussels)

• Laboratory exposure experiments
  – Sediment and sediment extract (Daphnia)
  – Selected CWA chemicals (blue mussels)

• Modeling leakage effects on marine biota & risk assessment
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Thank you

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