Impact assessment of chemical warfare agents from dumping sites in the Baltic Sea on the health of cod (Gadus morhua L.) and blue mussels (Mytilus edulis L.)

Brenner M., Baude R., Broeg K.

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Content

• Who are the CHEMSEA partners and associates?
• Structure of CHEMSEA project
• Goals and aims of CHEMSEA
• Outcomes of WP5 and contribution of the AWI
• Dumping history and examples of dumped warfare agents
• Sampling methods
• Preliminary results

Author contact information: Matthias.Brenner@awi.de
CHEMSEA - Consortium

Finland:
- Finnish Environmental Institute, SYKE
- Finnish Institute for the Verification of Chemical Weapon Convention, VERIFIN

Sweden:
- Swedish Defence Research Agency, FOI
- Swedish Maritime Administration, SMA
- European Center CBRNE of Umea University

Germany:
- Alfred Wegener Institute, AWI
- von Thünen Institute, vTi/FOE

Lithuania:
- Environmental protection Agency, EPA

Poland:
- Institute of Oceanology of Polish Academy of Sciences, IOPAS
- Military University of Technology, MUT
- Polish Naval Academy, PNA
CHEMSEA - Associated

- HELCOM MUNI, Helsinki, Finland
- Maritime Office Gdynia, Poland
- Sea Fisheries Institute, Gdynia, Poland
- Swedish Coastguard
- Ministries of Environment Finland & Sweden
- GIOS, General Inspectorate of Environment, Poland
- NRC, Vilnius, Lithuania
- Shirshov Inst. Of Oceanology, Russia
- Pomorskie Voivodship, Poland

Author contact information: Matthias.Brenner@awi.de
CHEMSEA - Workpackages

WP1 Management
WP2 Information
WP3. Invention of detection methods of CWA’s and their degradation products METHODS
WP4. Detection and characterization of dumpsites DETECTION

WP5 Ecological consequences of CWA & Risk Assessment ECO-RISK
WP6 Guidelines and contingency plans GUIDELINES

Author contact information: Matthias.Brenner@awi.de
CHEMSEA – Outputs (selection)

- Complete characterisation of Baltic chemical warfare agents (CWA) sites
- Recommendations on operating procedures for detection of underwater CWA objects and sampling in contaminated areas
- Calculation of costs of different leakage events
- Designation and guidelines for CWA contaminated areas
- Stationary leakage model
- Contingency plan

Author contact information: Matthias.Brenner@awi.de
CHEMSEA - Ecological consequences of CWA & Risk Assessment (WP5)

- Environmental effects & fate of contaminants
  - Exposure experiments
  - Biomarker analyses
  - Bioindicator based ecosystem state
- Ecological risk assessment and development of a leakage model

Author contact information: Matthias.Brenner@awi.de
CHEMSEA – Dumping History

• After World War II the Allies collected CWAs & conventional ammunition to disarm Germany
• Dumping was decided to be the most efficient and safest way of disposal
• Around 55 000 tons of CWAs were dumped into the Baltic Sea at the official dumpsites Bornholm Deep and Gotland Deep
• However there is evidence for CWA presence in many other places on the seafloor, due to wild dumping and displacement by sea currents and fishery activities
• Dumping practices continued during Cold War for decades (e.g. Gdansk Bay)

Author contact information: Matthias.Brenner@awi.de
### Dumped ammunition and CWA

Types of chemical munitions and quantities of warfare agents dumped east of Bornholm under control of the former Soviet Union (in tonnes)

<table>
<thead>
<tr>
<th>Type of warfare</th>
<th>Mustard gas</th>
<th>As-containing CWA</th>
<th>Adamsite</th>
<th>CAP</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aircraft bombs</td>
<td>5920</td>
<td>906</td>
<td>591</td>
<td>479</td>
<td>479</td>
<td>7896</td>
</tr>
<tr>
<td>Artillery shells</td>
<td>671</td>
<td></td>
<td>61</td>
<td>36</td>
<td>768</td>
<td>776</td>
</tr>
<tr>
<td>High-explosive bombs</td>
<td>314</td>
<td></td>
<td>65</td>
<td>36</td>
<td>42</td>
<td>314</td>
</tr>
<tr>
<td>Mines</td>
<td>42</td>
<td></td>
<td>924</td>
<td></td>
<td>924</td>
<td>924</td>
</tr>
<tr>
<td>Encasements</td>
<td>80</td>
<td>203</td>
<td>693</td>
<td>74</td>
<td>1050</td>
<td></td>
</tr>
<tr>
<td>Smoke grenades</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65</td>
</tr>
<tr>
<td>Containers</td>
<td>924</td>
<td></td>
<td>18</td>
<td></td>
<td>924</td>
<td></td>
</tr>
<tr>
<td>Drums</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td>18</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7027</strong></td>
<td><strong>2033</strong></td>
<td><strong>1428</strong></td>
<td>515</td>
<td>11077</td>
<td></td>
</tr>
</tbody>
</table>

**Author contact information:** Matthias.Brenner@awi.de
# CWA Types

<table>
<thead>
<tr>
<th>Type of chemical warfare</th>
<th>Chemical composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mustard gas</td>
<td>2,2’-Dichloro-Diethyl-Sulfide</td>
</tr>
<tr>
<td>Clark I*</td>
<td>Diphenylarsinchloride</td>
</tr>
<tr>
<td>Clark II</td>
<td>Diphenylarsincyanide</td>
</tr>
<tr>
<td>Adamsite</td>
<td>Phenarsazinchloride</td>
</tr>
<tr>
<td>Chloracetophenone (CAP)</td>
<td>2-Chloro-1-Phenylethanon</td>
</tr>
<tr>
<td>Phosgene</td>
<td>Carbonylchloride</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>Trichlormethane/ Chloroform</td>
</tr>
<tr>
<td>Tabun</td>
<td>P-cyano-N,N-dimethylphosphonamidsäureethylester</td>
</tr>
</tbody>
</table>

*Chlor-Arsen-Kampfstoff (Chloride-Arsenic-Warfare Agent)*

**Author contact information:** Matthias.Brenner@awi.de
Caused by e.g. altered cellular glutathione levels -utilizing this electron donor for the conversion of pentavalent to trivalent arsenicals or by oxidizing glutathione via arsenic-induced oxygen radicals (OH•, O2 •–)

oxidative stress

progressive and fatal cancer
hemic neoplasia gills, kidney, heart

(Pre)neoplastic changes hematopoietic tissue pathology

activation of redox-sensitive transcription factors and other signaling pathways involving p53 (protein level)

early carcinogenesis

Author contact information: Matthias.Brenner@awi.de
CHEMSEA – Sampling areas

~40 vessels (150 000 t of chemical warfare) at the depth 200–700 m
32 000 t at the depth of 70–105 m
2000 t at depth of 70–120 m

2 vessels with 69 000 tabun grenades at the depth 30 m (retrieved)

Official Dumping site
Wild dumping site
Semipelagic fishing
Reference site
Mussel caging

Wild dumping site unknown amount of conventional and chemical warfare
Blue mussel – *Mytilus edulis*

- Wild mussels from Bornholm shore were exposed from May until August 2012 at the dumping site Bornholm Deep in 35m and 60m depth
- Samples were taken for e.g.:
  - Chemical analysis
  - Histochemistry
  - Histopathology
- Project partners will analyse more than 15 different parameters in exposed mussels

Author contact information: Matthias.Brenner@awi.de
CHEMSEA – Fish sampling

Baltic cod – *Gadus morhua*

- 2 Expeditions in December 2011 and Mai 2012
- Semi-pelagic fishing at 2 dumping sites and 1 reference area
- Samples of blood, kidney, liver, muscle, bile and urine were taken for e.g.:
  - Histopathology
  - Histochemistry
  - Oxidative stress assessment
  - Chemical Analysis
- Project partners will analyse in total over 30 parameters per single fish

Author contact information: Matthias.Brenner@awi.de
<table>
<thead>
<tr>
<th></th>
<th>Head kidney</th>
<th>Kidney</th>
<th>Liver</th>
<th>Blood smears</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMS</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histopathology</td>
<td>x</td>
<td>x</td>
<td>x (vT1)</td>
<td></td>
</tr>
<tr>
<td>Neutral lipids</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Lipofuscin</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apoptosis (TUNEL)</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G6PdH</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Differential cell count</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>
Lysosomal Membrane Stability Test (LMS)

- The stability of the lysosomal membrane is measured as the time period required for its permeabilisation.
- The more stable the membranes are the longer is the destabilisation period.
- Toxic stress: membranes become less stable, risk of leakage of acid hydrolases and stored contaminants into the cytoplasm, risk of cell death.
- Biomarker for general toxicity reflecting toxically-induced alterations in various tissues like liver and hepatopancreas.

LMS-Test method:
- Snap-frozen tissue
- Serial sections
- Acid labilisation
- Substrate of acid hydrolase incubation
- Post coupling
- Staining of reaction product
- Image analysis

Author contact information: Matthias.Brenner@awi.de
CHEMSEA – First results

Lysosomal Membrane Stability

LMS-Test: Differences of the reference site (B09) are significant for both peak 1 and 2 (Kruskal-Wallis-Test, p<0.05, n=60)

Author contact information: Matthias.Brenner@awi.de
Histopathological Alterations

Head kidney samples were embedded in methacrylate, H&E stained and first analysed under LM (100fold magnification) for their general tissue architecture.

Reference site B09:
1. Blood vessel
2. Cluster of haematopoietic cells (HC)
3. Vacuolisation
4. Macrophage aggregates (MA)

Dumping site B13:
1. Blood vessel
2. Cluster of haematopoietic cells (HC)
3. Vacuolisation
4. Macrophage aggregates (MA)

Dense tissue, cluster of HC surrounding blood vessels, no vacuolisation, high prevalence of Small macrophage aggregates (MA)

Tissue less dense, HC cluster small with less number of cells, vacuolisation, depletion of small (MA), bigger MA more frequent

Author contact information: Matthias.Brenner@awi.de
Histopathological Alterations

Head kidney samples embedded in Methacrylate, H&E stained and analysed under LM (400fold magnification) according to:

1. Vacuolisation
2. Karyomegaly
3. Hypertrophy
4. Apoptotic bodies

Exposed sites

Reference site

Author contact information: Matthias.Brenner@awi.de
Histopathological Alterations

Number of histopathological alterations at all investigated sites.

* Differences between Dumping site B13 and Reference site B09 are significant (Kruskal-Wallis-ANOVA-Test, p<0.05, n=30)

Author contact information: Matthias.Brenner@awi.de
Thank you