ASSESSING THE BIOLOGICAL EFFECTS OF AMINES USED FOR CO_2-CAPTURE
- using the copepod *Calanus finmarchicus* as a model species -

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The *Reducing the Environmental impact of Acid gas Control Technology* (REACT) project

Ecotoxicity/biodegradation | Solvent characterization | Degradation mechanisms | Molecular modeling/QSAR | Process modeling

REACT – a multidisciplinary project at SINTEF Materials and Chemistry

Acid gas removal by absorption in aqueous solvents is a well-established process, but relies on large scale use of chemicals, especially alkanolamines. To identify environmentally friendly absorption chemicals for offshore natural gas CO_2 capture processes (pipeline transport and LNG manufacture), we characterized 43 amines in terms of biodegradability and ecotoxicity (standard OSPAR tests).

As very little is known about biological effects of amines, we have applied a molecular approach to understand biological modes of amine toxicity using a marine ecological key species (*Calanus finmarchicus*) as model organism.

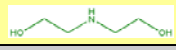
Calanus finmarchicus a marine ecological key species

- Estimated annual production of 300 mill. tons in the Northern Atlantic Ocean and Barents Sea (areas for future oil exploration)
- Important food for commercial fish species like Atlantic cod and herring
- High lipid content (up to 50% of body volume)
- The only continuous culture found at SINTEF/NTNU Sealab in Norway
- Recently this species has received increased attention as a relevant species for ecotoxicity testing and toxicogenomics

Selection of amines for biological effects assessment

- Amines are used for CO_2- and H_2S-removal (sweetening) of natural gas before further processing and are subject to discharges to the marine environment from offshore or coastal natural gas process plants
- According to Norwegian regulations for discharges from the oil industry, organic chemicals which meet two of the three following criteria are regarded as RED and should be phased out or substituted:
  - Biodegradability < 60%, or
  - Bioaccumulation potential Log Pow ≥ 5, or
  - Toxicity of EC50/LC50 ≤ 10 mg/L
- 43 amines were tested for biodegradation (BOD), bioaccumulation and acute toxicity (EC50) according to OSPAR Convention, and of these two were selected for assessing biological effects; one yellow and one red:

Diethanolamine (DEA)
- *Skeletonema* EC50: 356.9 mg/L
- BOD 44.8 %
- Bioaccumulation: 1.32
- *Calanus* LC50: 378 mg/L

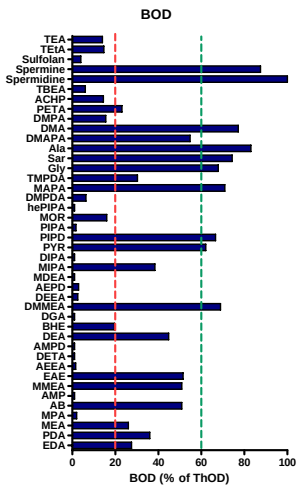


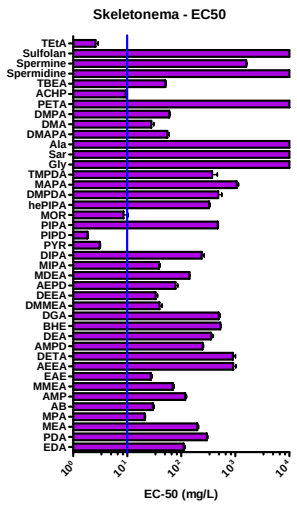
N-methyldiethanolamine (MDEA)
- *Skeletonema* EC50: 141.4 mg/L
- BOD < 1.0 %
- Bioaccumulation: 0.58
- *Calanus* LC50: 183.4 mg/L

Molecular approach

- Determine acute toxicity (LC_{50}) of DEA and MDEA for *C. finmarchicus*
- Suppression hybridization gene library generated from copepods exposed to DEA
- Annotation of expressed sequenced tags (ESTs) and separation of ESTs into Gene Ontology terms
- Conduct exposure experiments for DEA and MDEA with three concentrations (50, 5 and 0.5% of LC_{50}) for 12, 24 and 48 hrs
- Measure gene expression of ESTs putatively involved in essential biological mechanisms
- Assess time- and concentration-dependent relationships between amine exposure and gene expression
- Assess molecular modes of amine toxicity and indicate possible long-term effects of sub-lethal exposure

Biodegradability and ecotoxicity testing





Evaluation of chemicals in Norway is based on several tests, and a total of 43 amines were tested for biodegradation (BOD), acute toxicity (*Skeletonema costatum*), $LogK_{ow}$ and Microtox. Above are the results from BOD and acute toxicity.

For biodegradability, BOD is analyzed and BOD <60% is not accepted. In the figure accepted amines are found to the right of the red dotted line. Not accepted amines are to the left of the red line, indicating low biodegradability.

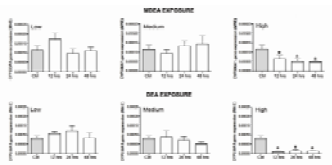
Acute toxicity is tested with the *Skeletonema* test, and for approval the amines must have EC50 > 10 mg/L. The accepted amines are to the right of the blue line in the diagram.

Two amines, one classified as red (MDEA) and one classified as yellow (DEA) were further tested for acute toxicity and biological effects on *C. finmarchicus*.

Quantitative real-time PCR (qPCR) for gene expression analyses

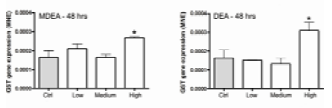
Gene expression of a series of genes were quantified using qPCR (Stratagene MX Pro 4005), and target gene expression was calculated using elongation factor 1α as reference gene.

Cytochrome P450 330A1



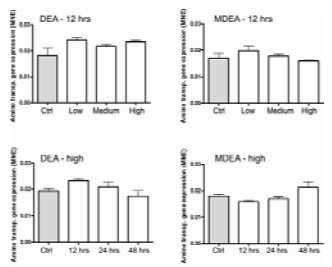
The CYP330A1 enzyme is involved in ecdysteroid and lipid metabolism. Its expression has been shown to be altered by naphthalene, water soluble fractions of oil and dispersed oil. We observed significant reduction in CYP330A1 gene expression at high concentrations of MDEA and DEA.

Glutathione S-transferase



GST has previously been found to be induced in *C. finmarchicus* after exposure to organic and inorganic compounds, indicating effects like lipid peroxidation. At high levels of MDEA/DEA we observed increased GST gene expression.

Amine transporter



The amine transporter was found in the SSH gene library as a putatively up-regulated gene. We did not observe significant induction of this gene following exposure to MDEA/DEA. The basal levels of amine transporter mRNA is quite high, and exposure-dependent induction of this gene may therefore be harder to observe. We also analyzed for Amine oxidase gene expression, and we did not find increased levels of this gene after exposure either.

Further reading about the molecular studies on Calanus finmarchicus:

Hansen, B. H., Altin, D., Hessen, K.M., Dahl, U., Breitholtz, M., Nordtug, T., Olsen, A. J. Expression of ecdysteroids and cytochrome P450 enzymes involved in lipid turnover and reproduction in Calanus finmarchicus (Crustacea: Copepoda). Gen. Comp. Endocrinol., in press.

Hansen, B.H., Nordtug, T., Altin, D., Booth, A., Olsen, A.J., Hessen, K.M. Expression of CYP330A1 and GST lipid-rich and lipid-poor Calanus finmarchicus following exposure to oil droplets and water soluble fractions of oil. Subm. to J. Toxicol. Environ. Health.

Hansen, B.H., Altin, D., Vang, S.-H., Nordtug, T., Olsen, A.J., 2008. Effects of naphthalene on stress gene transcription in Calanus finmarchicus (Crustacea, Copepoda). Aquat. Toxicol., 86, 157-165.

Hansen, B.H., Altin, D., Nordtug, T., Olsen, A.J. 2007. Suppression subtractive hybridization library prepared from the copepod Calanus finmarchicus (Gunn.) exposed to a sublethal mixture of environmental stressors. Comp. Biochem. Physiol., 2D, 250-256.

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