The reducing the environmental impact of acid gas control technology (REACT) project

Process modeling

Ecotoxicity/biodegradation
Degradation mechanisms
Molecular modeling/QSAR
Process modeling

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\textsubscript{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
- Seals 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\textsubscript{2} and H\textsubscript{2}S-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 handled or substituted:
  - Biodegradability < 60%, or
  - Bioaccumulation potential Log Pow 2.5, or
  - Toxicity of EC\textsubscript{50}/LC\textsubscript{50} 1 mg/L
- 43 amines were tested for biodegradation (BOD), bioaccumulation and acute toxicity (EC\textsubscript{50}) according to OSPAR Convention, and of these two were selected for assessing biological effects: one yellow and one red:
  - Deferoxamine (DEA) with EC\textsubscript{50}: 390.9 mg/L, BOD: 44.6 %, Bioaccumulation: 1.32, Calanus: 178 mg/L
  - N-methyldiethanolamine (MDEA) with EC\textsubscript{50}: 141.4 mg/L, BOD: 15.6 %, Bioaccumulation: 0.16, Calanus: 126 mg/L

### Molecular approach

- Determine acute toxicity (LC\textsubscript{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\textsubscript{50}) for 24, 48 and 48 hrs
- Measure gene expression of ESTs putatively involved in essential biological mechanisms
- Asses 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), Log\textsubscript{K\textsubscript{oW}} and Microtox. Above are the results from BOD and acute toxicity. For biodegradation, BOD is analyzed and BOD <40% 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 microalga Skeletonema and for approval the amines must have EC\textsubscript{50} > 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 1 as reference gene.

The CYP330A1 enzyme is involved in eutylol 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.

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.

The amine transporter was found in the SS5 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 Amines carboxagene expression, and we did not find increased levels of this gene after exposure either.

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