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N E W S L E T T E R F R O M

RF - Akvamiljø

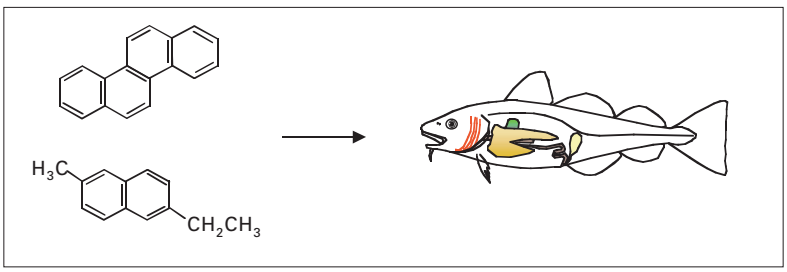
RF-Akvamiljø is a Centre for environmental research in aquatic environment. The three affiliated companies: RF-Rogaland Research, Akvamiljø a/s and Akvamiljø Caspian AS with 45 scientists and engineers provide advanced research and services within ecotoxicology, environmental risk assessment, monitoring, field- and laboratory experiments and analyses. Akvamiljø Caspian performs environmental consultancy/research in the Caspian region and operates a GLP standard laboratory in Baku, Azerbaijan

Determination of tar exposure to fish

A sensitive analytical tool is essential in order to determine polycyclic aromatic hydrocarbon (PAH) exposure to fish. Measurement of PAH metabolites in fish bile has proven to be such a sensitive monitoring tool. However, the metabolites can be determined by several analytical methods and the choice of method depends on the questions to be answered, the PAH compounds of interest and the required level of detail.

Tar compounds: Polycyclic aromatic hydrocarbons (PAH), commonly called tar compounds, are yearly released in large quantities to the marine environment. These compounds are absorbed by fish and efficiently metabolised to more water soluble compounds allowing excretion to take place. As a consequence, tissue levels of absorbed PAHs do not usually provide an adequate assessment of the exposure level. The main route for excretion of PAH metabolites is via the bile. As a result, analysis of PAH metabolites in fish bile constitutes a very sensitive method for determination of PAH exposure.

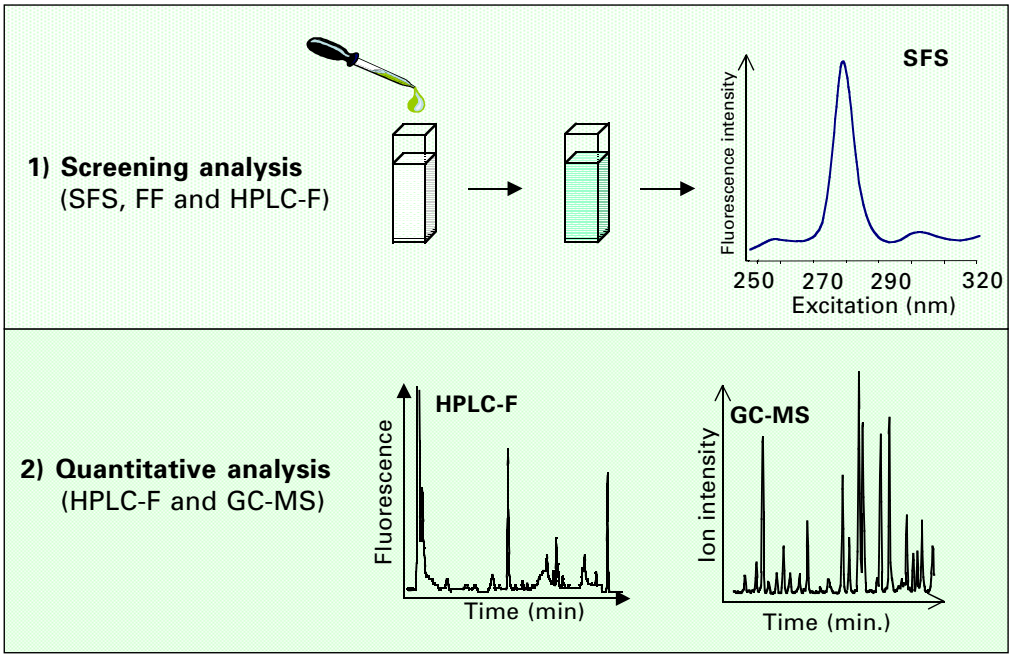
Exposure of fish to tar compounds



Analytical methods: PAH metabolites are commonly determined by semi-quantitative screening analysis or by quantitative determination of specific metabolites. Screening methods are very useful for rapid analysis of a large number of samples. The most frequent used screening methods are synchronous fluorescence scan (SFS), fixed fluorescence (FF) and high performance liquid chromatography-fluorescence (HPLC-F). These methods provide information about both pollutant source and exposure level. However, when the PAH metabolite level in fish bile are very low it may be difficult to distinguish between impacted and non-exposed individuals from the screening results alone. Quantitative analyses of specific metabolites have to be done in order to confirm the screening results.

Quantitative methods: A more sophisticated analytical procedure is required for quantitative determination of PAH metabolites. HPLC-F and gas chromatography connected to a mass selective detector (GC-MS) are the two most widely applied analytical techniques for quantitative analysis. The two methods have complementary advantages and disadvantages. Generally, GC-MS is the method of choice for analysis of the smaller and alkylated PAH compounds typically released with oil and produced water discharges. For determination of larger metabolites, HPLC-F would be preferred as analytical method.

Determination of PAH metabolites in fish bile; Analytical strategy



Analytical strategy: At RF-Akvamiljø we have established protocols for both screening (SFS, FF and HPLC-F) and quantitative analysis (GC-MS and HPLC-F) of PAH metabolites. A successive analytical strategy is recommended for the determination of PAH metabolites in fish bile. Initially, a simple screening method should be applied in order to determine the pollutant level and type of PAH contamination. When the screening results have been interpreted it should be decided weather it is necessary to go on with quantitative analysis for confirmatory analysis or not. The quantitative method to use depends strongly on the compounds of interest.

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RF-Akvamiljø is involved in the EU project "TRENDS"

RF-Akvamiljø is involved in the EU project TRENDS Thematic Network. The overall goal of TRENDS is to help to satisfy Europe's future demands and needs for sustainable, secure, safe and clean energy supplies, by identifying and addressing major challenges in the fields of Health, Safety, Environment and Quality (HSEQ). This will substantially contribute to meet the present and future needs of a sustainable European energy supply, with high quality and safety standards and minimised environmental impacts. This is particularly relevant as oil and gas will remain major energy resources for Europe until alternative, renewable energy infrastructures are in place. TRENDS has 64 partners and 6 observers from 13 countries. The project co-ordinator is Det Norske Veritas. The project comprises 11 work packages. Four work packages (WP1-WP4) are defined with expert groups within: Environmental Impacts, Health and Safety, Quality and Reliability and Social Responsibility. RF-Akvamiljø participates within WP1 with the responsibility to outline state-of-the-art within Environmental risk assessment.

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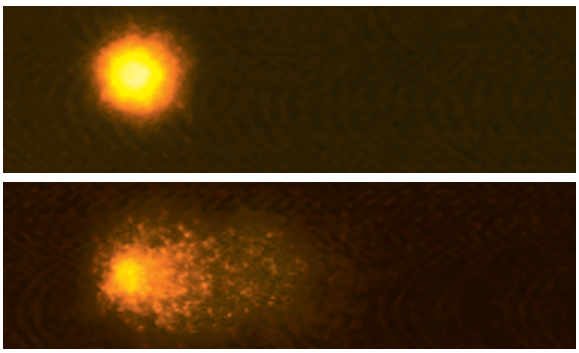
Detection of DNA damage in mussels following exposure to a bromated flame retardant

Polybrominated diphenyl ethers (PBDEs) is a large group of brominated flame retardants that have come to the attention of regulators and scientists because of their high potential for bioaccumulation in biota and persistence in the environment, together with their adverse health effects. PBDEs are close in chemical structure to PCBs (polychlorinated biphenyls) and they are commonly used in textiles, building materials and electrical equipment. Research has linked PBDEs exposure to an array of adverse health effects and they are suspected to be hormone disruptors, neurotoxic and can possibly also cause cancer, but data are scarce on the effect of PBDEs on marine organisms.

We have exposed mussels to the dominating PBDE congener found in biota (PBDE-47), at a concentration equivalent to ~0.05% of LC50 values found in literature. The comet assay was used to analyze DNA damaged in haemocytes (blood cells) and sperm from the mussels. The comet assay determines DNA damage in individual cells from experimental organisms exposed to potentially genotoxic compounds. The method measures the migration, during electrophoresis, of fragmented DNA away from the nuclei of cells immobilized in a thin gel. Examination of DNA damage is done under a fluorescence microscope after staining with a fluorescent DNA binding dye. The photographs show an undamaged (top) and a damaged cell (below) as seen under the microsope. The results are presented as the percentage of DNA migrated out of the nucleus and into the comet tail region.

For mussles exposed to PBDE-47, no effect was observed in blood cells, but in sperm cells, a statistically significant increase in DNA damage was found. The increased sensitivity of sperm could be related to that sperm cells do not have the ability to repair DNA.

Acknowledgements: The study was funded by the Norwegian Research Council (project no. 133724/420) and the European Commission through the BEEP project (EVK3-2000-00543).



Top: *Example of an undamaged cell with a nucleus consisting of a comet-head with minimal DNA migration into the comet tail region.*

Below: *Example of a damaged cell with a nucleus consisting of a comet-head with more DNA fragments having migrated into the comet tail region as a result of strand breakages.*

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BioSea JIP

ENI Norge AS and **Total E&P Norge AS** have joined forces regarding research on biomonitoring and biological effects, especially targeted towards Arctic and deep waters. Many of the two companies' activities at RF-Akvamiljø have been merged into a Joint Industry Project called BioSea JIP. Undoubtedly, environmental research benefits from co-operation. Therefore we welcome this development very much.

Two new Norwegian Research Council projects….

have been awarded to RF-Akvamiljø in the PROOF program concerning long term effects of discharges from the off-shore petroleum industry: 159183/720: Impacts of drilling mud discharges on water column organism and filter feeding bivalves,
Contact persons: Renee K. Bechmann / Stig Westerlund
Nr. 159176/720: Effects of off-shore oil industry related discharges in the Arctic,
Contact persons: Bodil K. Larsen / Thierry Baussant (pending for final approval)

What may *Eurythenes gryllus*, the giant deep-sea amphipod tell us….?

Biomarkers to monitor oil and gas industrial activities in the deep Norwegian Sea" – this is a 2-year Post doc. project which was initiated in 2002. It is supervised by Bjørn Gulliksen (University Center of Svalbard) and funded by the Norwegian Research Council, the oil industry and RF-Akvamiljø. Research is performed on Svalbard and the Akvamiljø Centre

Oxygen is essential for aerobic animals. However, when consumed oxygen generates potentially toxic compounds called reactive oxygen species (ROS). The ROS are responsible for DNA damages resulting in cell malfunctions (i.e. cancer). Therefore, living in an oxygenated environment has required the evolution of effective cellular strategies to detect and neutralize ROS in order to prevent damages. This strategy is based on a complex antioxidant system characterised by protein enzymes and small molecules (i.e. Vitamin C) that will scavenge ROS.

Numerous pollutants are reported to enhance the formation of ROS; hence, in ecotoxicology, measurements of antioxidant defences are widely used as biomarker of pollutant-mediated oxidative stress in marine invertebrates.

Although deep and Arctic Oceans are far way, contaminants originating from anthropogenic activities have been detected in the biota. For instance, the presence of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in deep-sea fish and invertebrates has been documented during the last decade.

Only few studies have focussed on deep-sea organisms and their capacity to metabolise pollutants. Furthermore, despite that contaminant metabolising enzymes were characterised in some deep-sea fish, to our knowledge, no investigations of the chemical biotransformation systems have been reported in deep-sea marine invertebrates.

In this study, the giant deep-sea amphipod *Eurythenes gryllus* was selected as sentinel species for measuring antioxidant defences. So far, three sampling expeditions in the deep Arctic Water have been carried out on board the research vessel "Jan Mayen"

International collaboration.

Dr. L. Camus from RF-Akvamiljø developed collaboration with Prof. Francesco Regoli (University of Ancona Italy), in order to implement a battery of assays to measure the antioxidant defences of marine organisms and to evaluate the impact of contaminants. Prof. Regoli has developed the Total Oxyradical Scavenging Capacity Assay (TOSC) which has brought new understanding on how marine organism adapt to oxidative stress.

Amphipod traps were built under the recommendation of Dr. Barry Hargrave (Bedford Institute of Oceanography, Dartmouth, NS, Canada)

.A presentation on "Antioxidant defences properties of deep-sea and Arctic amphipods" was made during the symposium "Pollutant Responses In Marine Organisms" (Tempa, Florida, US, May 2003)

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