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RF-Akvamiljø

Pollutant exposure and effects in fish related to the discharge of produced water in the North Sea oil industry

RF – 2004 / 192

Second annual project progress report

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Preface

This document constitutes the second annual progress report of the three year project "*Pollutant exposure and effects in fish related to the discharge of produced water in the North Sea oil industry*" (2003-2005) which is funded by the PROOF programme of the Norwegian Research Council. The participant institutions in the project are: RF-Akvamiljø, Rogaland Research, Stavanger (project leader); NIVA – Norwegian Institute for Water Research, Oslo; ITM - The Inst. of Applied Environmental Research, Stockholm; NTNU, Dept. of Zoology, Trondheim; Dept. of Chemistry, University of Florence (Italy), and the University in Stavanger.

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Stavanger, 26. October 2004

Jonny Beyer, project leader

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Populærvitenskapelig sammendrag

Forskere fra Norge, Sverige og Italia samarbeider i et treårig prosjekt for å bedre forståelsen av hvilke effekter som kan oppstå i fisk som følge av de kontinuerlige utslipp av produsert vann fra olje og gass produksjonen i Nordsjøen. Produsert vann inneholder mange forskjellige kjemiske forbindelser som miljøet helst skulle vært foruten, som for eksempel polyaromatiske hydrokarboner (PAH) og alkylfenoler. Normalt er heldigvis den faktiske mengden av PAH og AP relativt liten i produsert vann, men stadig flere olje og gassfelt når nå en sen produksjonsfase og mengden produsert vann øker da vesentlig.

Miljøundersøkelser av denne type setter store krav til forskerne og de metodene de bruker. Havet er stort og stoffene som slippes ut med produsert vann blir raskt fortynnet i vannmassene. Forskerne er derfor særlig interessert i om effekter av PAH, alkylfenoler og andre stoffer fra produsert vann kan oppstå i fisk ved vedvarende eksponering ved svært lave konsentrasjoner. For at det skal være mulig å måle opptak og effekter av produsert vann substanser i fisken har forskerne gått nye veier for å gjøre målemetodene bedre. Et vesentlig bidrag til dette arbeidet ble nylig levert av to studenter fra (det kommende) Universitetet i Stavanger. Gjennom sine hovedoppgaver var studentene med å utvikle målemetoder for alkylfenol metabolitter i fiskegalle, en målemetode som forskerne håper skal gjøre det mulig å påvise om fisk som finnes på olje og gass feltene i Nordsjøen blir eksponert for svært lave konsentrasjoner av alkylfenoler fra produsert vann utslipp. Dette ville i så fall bety en banebrytende forbedring av miljøovervåkingsverktøyet for alkylfenoler i Nordsjøen.

Slike lave (og vedvarende) eksponeringskonsentrasjoner kan i visse tilfeller gi forskjellige typer uheldige effekter i fisk, som for eksempel akkumulering av genetiske skader (i tilfelle av PAH) og forstyrrelser av visse hormon styrte prosesser (i tilfelle av visse alkylfenoler). Disse forhold blir også studert i dette prosjektet, og også her må forskerne utføre nybrottsarbeid for å bedre sensitiviteten av metodene. I tillegg til mer sensitive målemetoder prøver forskerne også å utvikle billigere og raskere metoder for miljøovervåkingen. Her har de italienske prosjektdeltagerne sin styrke. De kan utvikle billige bio-sensorer som kan muliggjøre hurtig og billig analyse av mange fiskeprøver. Forskerne ønsker dessuten å benytte en avansert protein analysator (en såkalt proteomics maskin) til å lete etter effekter som vi hittil ikke har kjennskap til. Dette arbeidet skal utføres nå i det siste året av dette treårige prosjektet.

Ta gjerne kontakt med forskerne ved RF-Akvamiljø (www.rf.no/internet/akva.nsf) dersom du er interessert i mer informasjon. Institusjonene som deltar i prosjektet er RF-Rogalandsforskning (prosjektleder); Norsk Institutt for Vannforskning (NIVA); ITM-Laboratoriet (Universitetet i Stockholm); NTNU Trondheim, Universitetet i Firenze, og Universitetet i Stavanger.

1 Background

The offshore oil industry in Norway is currently in a period during which the amount of produced water (PW) is increasing. The Norwegian Research Council has recognised the need for better knowledge about effects of PW and other dischargers from the oil production in marine biota. A separate program (PROOF) was launched to address this topic specifically. Among the research needs; the definition of relevant and operational sets of biological effect parameters, the development and refinement of effect-monitoring tools and the performing of field studies in PW affected areas have been emphasised. The present project is sponsored by PROOF in the period 2003-2005 (Table 2) and is functionally an extension of the two NFR projects 152231-720 & 152449-720 that were conducted in 2002-03.

Table 1: Overview of project work packages and the initial time plan.

6 Milestones – timetable for the project															
Project period: From:	01.01.03	To:	31.12.05	2003				2004				2005			
Milestones and principal activities				1	2	3	4	1	2	3	4	1	2	3	4
WP-1) Biological exposure samples of fish				x		x				x					
WP-2) GC/MS AP metabol. bile (post-doc)						x	x	x	x	x	x	x			
WP-3) ICP-MS PW metals in bile					x	x									
WP-4) Genotox biosensor bile					x	x				x					
WP-5) Genotox stress in liver						x	x			x	x	x	x		
WP-6) Endocrine, embryo and develop effects					x	x	x	x	x	x	x	x	x		
WP-7) Effects thyroid horm. and vitamins						x	x				x	x	x		
WP-8) Proteomics study							x				x	x	x		
Project meetings				x		x			x			x		x	
Adm., reporting, dissemination, publishing				x	x	x	x	x	x	x	x	x	x	x	x

2 Project objectives and time-plan

The present project has three major objectives. Our first aim is to provide relevant sample materials from fish exposed in the laboratory to PW components (crude oil, petrogenic PAHs, petrogenic alkylphenols). Such samples are needed both for the development of new techniques but also for optimisation of existing methods, and as a basis for the interpretation of analytical results of fish collected in PW affected areas (e.g. the Tampen region). Atlantic cod (*Gadus morhua*) is the prioritised study species in this project, but also other gadoid species, such as haddock (*Melanogrammus aeglefinus*) and Saithe (*Pollachius virens*), may be included in some of the project studies.

The second major project aim is to study and describe the potentials of certain constituents in fish bile as monitoring parameters for the assessment of PW exposure in wild fish. In details, this work includes: (a) the development of a GC-MS based analysis of alkylphenol metabolites in bile from exposed fish; (b) refinement and validation of ICP-MS detection of bile metal constituents from exposed fish; and (c) the development/refinement of a DNA biosensor assay to allow a more rapid detection (screening) of genotoxic exposure in exposed fish. The bile fluid is one of our major targets since this complex biotic fluid is the prime route for excretion of many pollutants in fish (and other vertebrates) and since it virtually is a natural extract sample that reflects the recent and ongoing exposure to a range of pollutants. Samples from exposure studies in the lab as well as samples of fish collected in the field (e.g. Tampen area) will be used in these studies.

The third main objective of the present project is to investigate sub-lethal biological effects that can be induced in PW exposed fish. In details, our emphasis is put on: (a) hepatic biomarkers of genotoxic stress; (b) endocrine related effect parameters in fish plasma that may signal long-term reproductive and developmental impact; and (c) the potential of using novel proteomics detection methods as a mean for revealing new pollution effect phenomena in PW exposed fish. Biological samples from exposure studies in the lab as well as samples of fish collected in the field will be used in these studies.

3 Status of project work packages

3.1 WP-1: Fish samples for PW effect assessment

Work-package responsible: RF-Akvamiljø

The objective of WP-1 is by various means to provide relevant samples to the other work packages of the project. The samples needed are primarily biological sample materials from fish exposed (in the lab) to dispersed crude oil and to PW-relevant alkylphenols and PAHs (single component and mixture exposures). Fish exposures in the lab should include both high dose, low dose & control treatments. In addition, samples of fish collected from relevant offshore locations, such as the Tampen field, are needed for field validations of effect parameters.

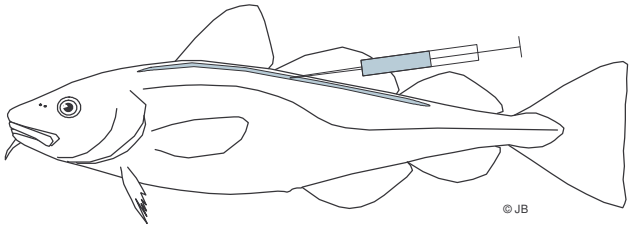

Figure 1: The new fish exposures with single APs in 2004 employed the same simple exposure technique as used in last year exposures. The fish were exposed by means of injecting AP compounds into the area between the two major axial muscles. The exposure agents were first solved in acetone and subsequently transferred to the cod-liver oil vehicle.

Several new fish exposures have been carried out in the present project and in NFR-152449/720; the latter project was a pre-study of WP-2 (see separate section of WP-2 below). Primarily the fish exposures were carried out in order to make positive reference materials of Atlantic cod exposed to PW relevant PAHs and APs. In these laboratory exposures, the use of intraperitoneal injection was omitted in order to avoid contaminating the biological samples (e.g. liver) directly with exposure agent. Instead the injection was given subcutaneous/inter-muscularly on the dorsal side of the fish (Figure 1). The set of injection exposures has been conducted at RF-Akvamiljø are shown chronologically in the tables below.

Table 2: The concentration of AP exposure solutions and the injected doses which were used for exposure of cod; during December 2002 and May 2003, respectively in the NFR-152449/720 project.

	Carrier concentration	Injected dose in fish
4-methylphenol (para-cresol)	10 mg/ml	10 mg/kg
2-methylphenol (ortho-cresol)	10 mg/ml	10 mg/kg
3,5 dimethylphenol	10 mg/ml	10 mg/kg
2,4,6 trimethylphenol	10 mg/ml	10 mg/kg
para-tert-butylphenol	1 mg/ml	1 mg/kg
4-tert-butyl-2-methylphenol	10 mg/ml	10 mg/kg
AP mixture	10 mg/ml	10 mg/kg

Table 3: The exposure concentrations of methyl PAHs used for single compound inter-muscular injection exposure of Atlantic cod in January 2004.

Compound	Abbreviation	purity	Weight PAH	Weight codliver oil	Conc
		%	mg	Ml	mg/ml
2-methylnaphthalene	2-me-NPH	95-97	51.38	5.1	10.07
2-methylphenanthrene	2-me-PHE	99.9	8.71	4.35	2.00
5-methylchrysene	5-me-CHRY	99.8	8.92	4.5	1.98
codliveroil	Control				

Table 4: The exposure concentrations of alkylphenols used for single compound and mixture exposures of Atlantic cod in September 2004.

Compound	Abbreviation	purity	Weight PAH	Weight codliver oil	Conc
		%	mg	ml	mg/ml
4-tert-butylphenol	4-tert-C4-phenol	99.5	50.0	5.0	10.0
4-n-pentylphenol	4-n-C5-phenol	99.0	50.8	5.1	10.0
4-n-hexylphenol	4-n-C6-phenol	99.8	57.5	5.8	9.9
4-n-heptylphenol	4-n-C7-phenol	98.5	86.0	8.6	10.0
AP mixture	mix of 9 AP	-	134.9	10.0	13.5
codliver oil (Møllers Tran)	Carrier control	-	-	-	-
control	-	-	-	-	-

Table 5: Exposure concentration of alkylphenols in the AP mixture group of the exposure study conducted in September 2004 (referring to the AP mix group in Table 4.

	Abbreviation	purity	Weight PAH	Weight codliver oil	Conc
		%	mg	ml	mg/ml
2-methylphenol	2-MP	99	14.6	10.0	1.5
4-methylphenol	4-MP	99	14.1	10.0	1.4
3,5-dimethylphenol	3,5-DMP	98	14.9	10.0	1.5
2,4,6-trimethylphenol	2,4,6-TMP	99	15.0	10.0	1.5
4-tert-butylphenol	4-tert-C4-phenol	99.5	21.3	10.0	2.1
2-methyl-4-tertbutylphenol	4-t-2M-C5-phenol	≥ 98	13.4	10.0	1.3
4-n-pentylphenol	4-n-C5-phenol	99.0	12.6	10.0	1.3
4-n-hexylphenol	4-n-C6-phenol	99.8	15.1	10.0	1.5
4-n-heptylphenol	4-n-C7-phenol	98.5	13.9	10.0	1.4
Sum AP			134.9	10.0	13.5

Presently coming up in WP-1 is an advanced exposure study which is to be conducted at RF-Akvamiljø in November 2004 in collaboration with the Institute of Marine Research. In this exposure study, Atlantic cod will be exposed to various concentrations of real produced water in the laboratory as well as to dispersed crude oil and mixtures of alkylphenols (for details see appendix 1).

Several of the work packages in the present project have utilised existing fish sample materials from other projects, see table 2 in the previous project report for an overview of relevant materials.

3.2 WP-2: GC/MS detection of alkylphenol metabolites in bile

Work-package responsible: RF-Akvamiljø

The objective of this WP is to develop and optimise procedures for determination of alkylphenol (AP) metabolites in fish bile. Pilot feasibility studies were in 2002-2003 carried out in the PROOF funded pre-project NFR-152449/720 (xxx ref), and a continued testing and method optimisation program is now carried out in WP-2 of the present project. The post doc of Dr.scient Grete Jonsson (one man-year split over two years) constitutes WP-2 The method development and testing activity include studies of alternatives related to sample cleanup procedures, deconjugation and derivatisation of AP metabolites and separation, identification and quantification by means of GC/MS-SIM.

In 2004, GJONs post doc activity also included the supervision of two master students, Admira Cavcic & Tone Ulland Stokke, from the Stavanger University College. Dr. Assoc. prof. Kåre B. Jørgensen was the supervisor situated at the Stavanger University College. The two master theses were finished in June 2004 (see appendix 2 and appendix 3). Initially, the method development for alkylphenol metabolite detection was focussed technically on GC/MS-SIM. In the two master theses, a HPLC approach of the method development was addressed. This was an extra approach which not was included in the initial project description. The abstracts of the two master theses are as follows:

Admira Cavcic, M.Sc. study - 2004

Abstract: Six alkyl phenol metabolites were determined by high performance liquid chromatography connected to a fluorescence detector. Limits of detections (LODs) for 4-methyl-, 2-methyl-, 3,5-dimethyl-, 2,4,6-trimethyl-, 4-tertbutyl- and 4-tertbutyl-2-methylphenol were 1.5 ± 0.2, 2.6 ± 0.9, 0.5 ± 0.1, 0.6 ± 0.1, 1.1 ± 0.2 and 0.8 ± 0.1 ng/g, respectively. Two different sorbets, Phenyl and ENVI-Carb, were compared for solid phase extraction of alkyl phenols in fish bile. Higher recoveries and lower matrix interference were obtained using the ENVI-Carb column. Five of six alkyl phenols were well separated from endogenous bile compounds, and overall LODs were ranging between 16-41 ng/g bile. The developed method was applied to the analysis of deconjugated alkyl phenol metabolites in bile from fish exposed to single compounds in the laboratory. Large amounts of the respective metabolites were detected. In order to improve the sensitivity of the method alkyl phenols were derivatized with dansyl chloride. LODs for those six alkylphenols were ranging between 0.02 - 0.04 ng/g, which improves the sensitivity with a factor 10. However, the derivatized alkyl phenols were not separated by use of the available analytical column (Reverse phase C_{18}).

Tone Ulland Stokke, M.Sc. study - 2004

Abstract: A reversed phase HPLC-F method that separates and detects six alkylphenols in fish bile has been established. The six alkylphenols were 4-ethylphenol, 2-

methylphenol, 3,5-dimethylphenol, 2,4,6-trimethylphenol, 4-tertbutylphenol and 4-tertbutyl-2-methylphenol. The method involves a gradient run with a mobile phase consisting of two methanol-water buffers of different polarity. Triphenylamin was used as internal standard. Two different Solid Phase Extraction (SPE) cartridges, C-18 and Envi-Chrom, were tested for sample treatment prior to HPLC. Both the cartridges yielded high recoveries and removed the same degree of interferences. The C-18 cartridge was chosen because of a lower price. Overall Limits of Detection for the alkylphenols in the bile matrix varied between 40 and 160 µg/g. Utilisation of the established method on lab exposed fish showed that the method was able to detect all the six alkylphenols in bile taken from fish exposed to single compounds. The same exposure level resulted in higher concentrations of the larger alkylphenols (3 – 5 carbons attached) as compared to the smaller (1 – 2 carbons attached). The amount of alkylphenols present in the bile in unconjugated form was investigated, and proved to be close to or below the detection limits. The established HPLC method was applied to bile samples from fish caged in four different locations offshore, at increasing distances from an oil platform. The samples from the group sited closest to the platform were contained one compound eluting very close to 4-tertbutylphenol. Spiking with a standard proved that it was not the C_4 alkylphenol isomer used in this project. This compound was found in decreasing amount with increasing distance from the platform. Another compound that probably is 4-tertbutyl-2-methylphenol was also detected in the samples from the two groups closest to the platform. However, the peak was very small and appeared as the left shoulder of a slightly larger peak, and was therefore not possible to identify positively.

The possibility of using the less demanding HPLC approach as a tool for AP-metabolite detection is very interesting but limited to the analyses of simple exposures of known compound. Alkylphenol metabolites in bile taken from fish exposed to environmentally exposed fish could not be positively identified by HPLC-Fluorescence (Figure 2).

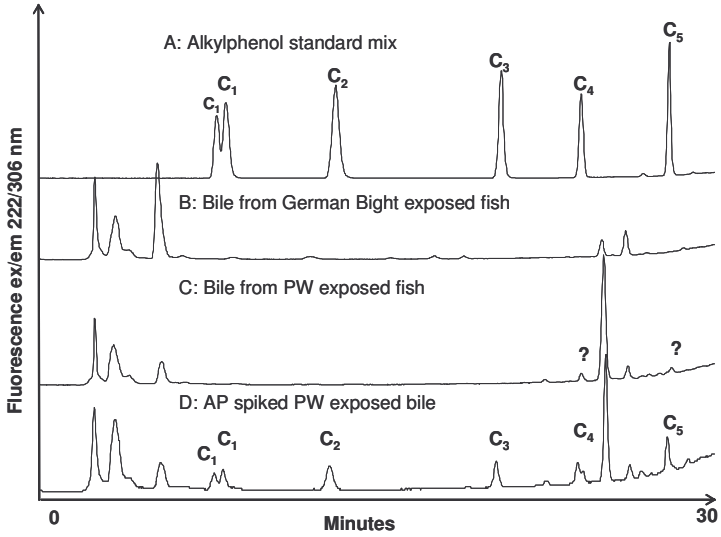

Figure 2: HPLC-F chromatogram of a mixture of A) six alkylphenols B) bile from fish caged in the German Bight, C) bile from fish caged outside the Statfjord B oil platform and D) sample C) spiked with six alkylphenols.

However, the results obtained by the two students and the continuation of their work have enabled us to propose a metabolic route for alkylphenols absorbed by fish (Figure 3 & Figure 4). These results will be submitted as a manuscript to Environmental Toxicology and Chemistry

within 2004. GC-MS constitute a more powerful analytical technique with respect to separation capacity, sensitivity and possibility for identification of AP metabolites. We have so far established a GC-MS method for which we have determined the instrumental detection limits for derivatised and non-derivatised alkylphenols. Separation performance and detection limits were improved for derivatives, and BSTFA has been shown to be a more efficient reagent as compared to TMSI. Continued test-runs with AP standards and bile samples from fish exposed to individual alkylphenols (see WP-1 section) have been analysed in 2004, and this activity will be continued in 2005. The analyses in 2005 will also be conducted with bile samples from the cod which are to be exposed to produced water and other complex matrices in the RF-Akvamiljø laboratory in November 2004. Ultimately the feasibility of the optimised method will be validated with samples from fish collected at relevant production fields in the North Sea. And the sensitivity of the GC/MS metabolite detection will be compared to chemical detection of AP parent compounds in liver. The experience and results gained until now in WP-2 were presented at the PROOF annual meeting at Bårdshaug Herregård in Orkanger in October 2004. It is also a prioritised task for 2005 to ensure a peer reviewed publication of the developed GC-MS method.

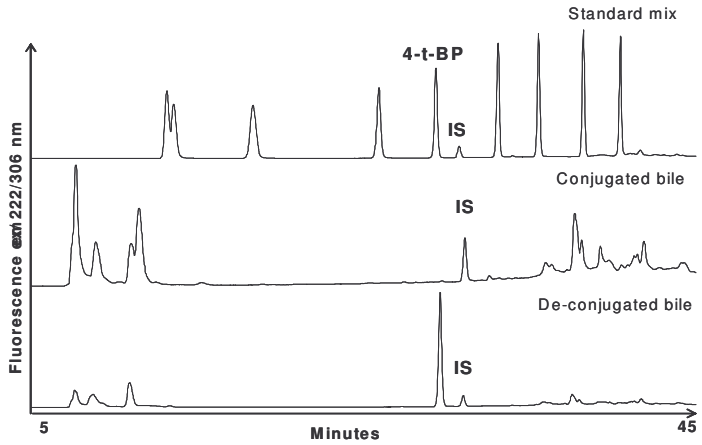

Figure 3: HPLC-F chromatogram of a mixture of nine alkylphenols, conjugated and de-conjugated metabolites in bile from cod exposed to 4-tert-butylphenol

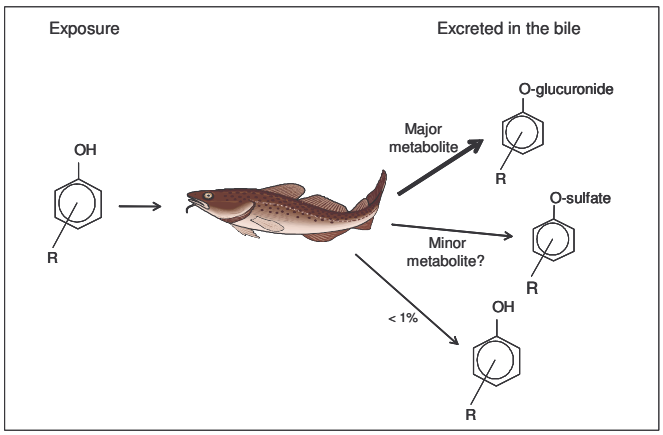

Figure 4: Metabolites excreted in fish bile following inter muscular injection of alkylphenols

3.3 WP-3: ICP-MS detection of PW metals in bile

Work-package responsible: RF-Akvamiljø

This is a smaller work-package in the present project. The objective is to provide an evaluation and field validation of an ICP-MS method for detection of PW related metal contamination in fish bile. It has not been new activity in the WP since last progress report. See previous progress report for more information.

3.4 WP-4: DNA-biosensor detection of bile genotoxicity

Work-package responsible: Univ. of Florence, Italy & RF-Akvamiljø (Thierry Baussant)

The main objective of this WP is to investigate the feasibility of using biosensor based approach combined with bile analyses as screening tools to examine pollutant stress in fish. Most activity so far has been concentrated on a DNA biosensor assay which has been developed by Prof. Marco Mascini and his colleagues at the University of Firenze (see previous progress report). By a collaboration of the Mascini group and RF-Akvamiljø, this biosensor assay has been tested with fish samples produced in laboratory exposure studies as well as with fish materials collected at offshore fields as well as in an PAH contaminated coastal recipient (Karmsundet). This activity is also sponsored by TOTAL E&P Norge. The results from these studies are presently being published and have also been presented at several scientific symposia; e.g. as recently at the Euroanalysis XIII in Salamanca (Spain) 5-10 Sept. 2004 (see abstract below).

Abstract to Euroanalysis XIII

Assessment of environmental pollution in the North Sea through the analysis of fish bile with DNA-based biosensor

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DNA based biosensors represent a new research field with interesting possibilities for practical application in various fields such as environmental and medical screening. Many molecules show a high affinity for DNA and they can interact with the nucleic acids immobilised on the electrode surface. The interactions between DNA and environmental pollutants can cause chemical and conformational modifications of nucleic acids and thus variation of the electrochemical properties of DNA. The presence of these compounds is measured by their effect on the guanine base: the changes in oxidation of the guanine peak, obtained by a square wave voltammetric scan, is used as analytical signal [1].

Measurement of contaminants or their metabolites in tissues or body fluids of organisms were exploited in this research. A rapid and low cost device, based on electrochemical DNA biosensor, is proposed as a screening tool for the detection of PAH exposure at contaminated sites. Laboratory studies have demonstrated that the presence of PAH metabolites in bile is well correlated with levels of exposure [2]. The gallbladder bile is a major excretion route for PAH in fish. After biotransformation, PAH metabolites are excreted into the bile and concentrated. Thus, the bile can be used as an indicator of PAH exposure.

Preliminary studies with PAH metabolites standard solution were performed, using naphthalene, phenantrene, pyrene and benzo[a]pyrene hydroxy or dihydroxy as model compounds. The effect of these compounds on the surface-confined DNA was found to be linearly related to the concentration of the PAH derivative in solution. Then, the DNA biosensor was used to investigate the genotoxic effect due to the presence of PAH metabolites in fish bile samples. Two different sample groups were analysed: bile analysis of fish injected with a single PAH and fish caught bile samples at different sampling sites in the North Sea. A one-way analysis of variance (ANOVA) was used to compare between the different sample

groups. When the ANOVA indicated that significant differences existed, Fisher´s multiple comparison test was then used. Dunnett's test was used to determine the significance of differences between the reference sample groups and the control (blank assay) group. A significance level of $P<0.05$ was applied in all statistical tests. The results demonstrated that the sampling sites presented different pollution levels and that the PAH derivative presented different genotoxic effect.

The results obtained with the biosensor analysis were compared with fixed wavelength fluorescence (FF) at the excitation/emission wavelenghts of pyrene (340/380nm) and benzo[a]pyrene (380/430nm) and a good correlation was found.

[1] Lucarelli F., Authier L., Bagni, G., Baussant T., Aas E., Marrazza G., Mascini M., *Anal. Lett*., 36, 9, 1887-1901 (2003).

[2] Lin E.L.C., Cormier S.M., Torsella J., *Ecotox. and Env. Safety*, 35, 16-23 (1996).

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In addition to the above described biosensor, another biosensor assay which also has been developed by the Mascini group has in 2004 been tested for its applicability as a screening tool in relation to PW monitoring issues. This is a peroxidase based amperometric biosensor and it has been tested for its applicability for detection of PW relevant alkylphenol metabolites in fish bile samples. Emily Bulukin from the Mascini group has conducted the analyses and the samples used in the test are bile from AP exposed cod (see WP-1). The study report of this work is included in the appendix (see appendix 4).

3.5 WP-5: Hepatic biomarkers of PW genotoxicity in fish

Work-package responsible: ITM Univ Stocholm (Lennart Balk)

In this WP, the main aim is to improve the method for detection of hepatic DNA adducts in fish exposed to PW components in laboratory exposures and in fish collected at PW relevant field locations (e.g. Statfjord-Tampen field). A method development section on optimisation of the DNA-adduct 32P-postlabelling assay towards a typical oil (and PW) related PAH profile (high content of naphthalene and phenanthrenes and alkylated PAHs) is to be conducted. The assay developments of DNA adduct detection for smaller and alkylated PAHs means roughly to optimize and improve TLC separations for these kinds of structures, implying a 2-3 month optimising study at ITM.

In addition, the detection of DNA adducts in mitochondrial DNA instead of in nuclear DNA will be investigated. The aim is to evaluate the mitochondrial adducts as a possible more sensitive biomarker towards petrogenic DNA adduct forming agents. Further information about the potential related to mitochondrial DNA adduct detection in fish is provided in a note by Skarphedinsdottir and Balk (see appendix 5).

For the method development liver samples of cod exposed to a range of single PAHs that are relevant to crude oil (naphthalene, phenantherene, fluorene, dibenzothiophene, chrysene, and smaller alkylated PAHs) have been submitted from RF-Akvamiljø to the ITM lab in Stockholm. In addition, Samples for this purpose will be obtained from the PW exposure study at RF-Akvamiljø in November 2004.

Unfortunately, the analyses and method development in WP-5 has been severely delayed as compared to the initial time-plan due to major technical difficulties with the 32P-postlabelling assay at ITM. Consequently, the time-plan of this WP has to be rearranged, implying that parts of the resources allocated for WP-5 in the earlier phase of the project must be transferred to 2005.

3.6 WP-6: Endocrine, embryonic and developmental effects of PW in fish

Work-package responsible: NIVA & ITM lab-University Stockholm

The objective of this WP is to clarify whether exposure of fish to PW under field or laboratory conditions affects estrogen-sensitive biomarkers, i.e. vitellogenin, zona radiata proteins and steroid-binding proteins, selected enzymatic pathways in the metabolism of steroid hormones in female gonads ex vivo, as well as the development of fish larvae. The steroid profile will be assessed in the bloodplasma of selected fish, including pregnenolone, progesterone, 17a-hydroxyprogesterone, androstenedione, estrone, 17ß-estradiol, testosterone, 5a-dihydrotestosterone, 3a17ß-androstanediol, adrenosterone, 11-ketotestosterone, 17a 20ß-dihydroxy-4-pregnen-3-one, 11-deoxycortisol and cortisol. Effects on fish embryonal development will be assessed through simulated maternal transfer, i.e. egg nanoinjection. Selected components in produced water and total extracts of produced water will be assessed through this method. Comparative studies will be done using salmonid and gadoid eggs and embryos. In addition to embryonal development, selected biomarkers will be assayed in the developed larvae.

The activity in WP-6 so far in 2004 have been concentrated on a study by Knut-Erik Tollefsen (NIVA) of chemical modulation of sex steroid binding proteins (SBP) in saithe collected at the Statfjord, Sleipner and Egersund fields during the 2002 Tampen survey and in Atlantic cod deployed during the water column survey at different distances from the Troll B platform in 2003. Although the effect of direct interference with and modulation of circulating levels of plasma SBP has not been properly elucidated, several possible mechanisms of endocrine disruption have been proposed (Figure 5). Direct interaction with the SBP and modulation of SBP properties, either singly or in complex mixtures, may represent novel mechanisms for endocrine disruption and explain why weakly acting endocrine disruptors like the phthalates are causing "estrogen-like" reproductive disturbances in developing males without exerting their action through the estrogen receptor. An apparent reduction in SBP binding capacity was found in saithe collected in the Statfjord area, compared to the Egersund area (Figure 6 - upper fig). A similar effect was seen in cod at the 2000m station during the 2003 Water column survey (Figure 6 – lower fig) in the male fish. Statistics will be run to determine the significance of the data, but data from some groups are hampered by low sample numbers, thus reducing the resolving power (see appendix 6 for more details about this study).

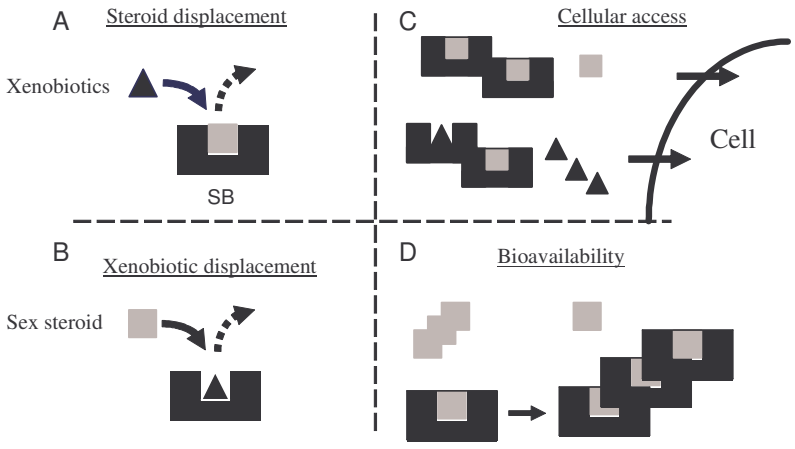

Figure 5: P*roposed mechanisms for chemically induced interference and modulation of sex steroid-binding protein (SBP) function: A) exogenous ligands (xenobiotics) are able to displace potent endogenous sex steroids (estrogens and androgens) and increase cellular access, B) high concentrations of locally produced sex steroids in gonads are able to displace xenobiotics and lead to tissue-specific accumulation, C: differential binding of SBP ligands leads to enhanced cellular access and potency of*

weak SBP binders and D) increase/decrease in circulating levels of SBP leads to decrease/increase in the bioavailable fraction of potent sex steroids.

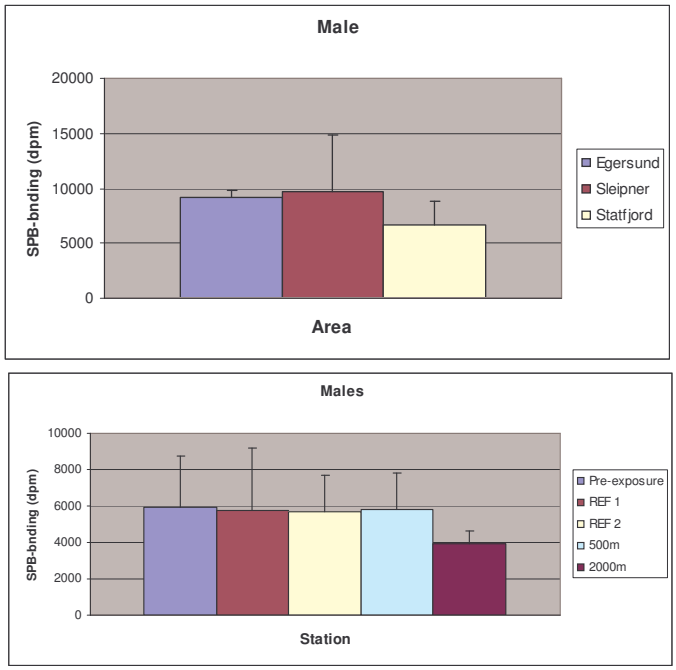

Figure 6: Upper figure: Total SPB binding capacity in plasma from male Saithe caught in the Egersund, Sleipner and Statfjord area (n=4-10). Lower figure: Total SPB binding capacity in plasma from Atlantic cod deployed during the water column survey at different distances from the Troll B platform (n=6-10). (see report by Tollefsen in appendix 6 for more details about these results).

3.7 WP-7: PW effects on thyroid hormones, steroids, vitamin homeostasis and behaviour in Atlantic cod

Work-package responsible: NTNU

The objective of this WP is to address effects of PW constituents on thyroid hormone systems and on vitamin A, E and B in fish. Thyroid hormones (total and bound thyroxin and triiodothyronine) and vitamin A, E and B will be measured in selected samples from fish exposed in the lab or in fish collected during field surveys (Tampen). WP-7 is a smaller work-package in the present project, and activity has not yet been launched due to lack of relevant samples. The present plan is to use samples obtained from the PW exposure study in November 2004 as the main sample material for addressing this WP.

3.8 WP-8: Proteomics analyses of PW exposed fish

Work-package responsible: RF-Akvamiljø

The objective of this WP is to carry out a Proteomics based pilot study on fish exposed to PW components. A Surface-Enhanced Laser Desorption Ionisation Time of Flight (SELDI-TOF) unit has been implemented at RF-Akvamiljø and this instrument will be used in the study. The analytical activity will primarily be conducted with the samples obtained from the PW exposure study in November 2004 and this is well in accordance with the initial time-plan.

4 Project meetings

- **6/2 2003:** Project kick-off meeting with RF-Akvamiljø project personnel only.
- **24-25/03 2003:** A two-day project inception meeting at RF-Akvamiljø with all project partners and RF-Akvamiljø project personnel.
- **19/8 2003:** Meeting with most project partners and project collaborators at NIVA, Oslo.
- **4/11 2003:** Project progress meeting with RF-Akvamiljø project personnel only.
- **27 feb-04:** Project progress meeting with RF-Akvamiljø project personnel only.
- **17-18/06 2004:** A two-day project meeting at RF-Akvamiljø with most project partners and all involved RF-Akvamiljø project personnel.

5 Reporting

The reporting of the present study will follow the ordinary NFR system (progress reporting and project end report).

6 Dissemination and publication

In this project it is prioritised to disseminate the project to relevant user groups, including oil industry agencies, scientific task groups (e.g. ICES) as well as to environmental regulatory bodies (OSPARCOM and SFT), science students and other researchers as well as to the public. This aim will be accomplished through presentation activities at relevant seminars, meetings and conferences as well as through publication of results in scientific journals and by popular dissemination to the public through appropriate phora and media. In several of the work-packages (WP-2 and WP-4) there are manuscripts being prepared for peer review publication of results from the present project. Several manuscripts are expected to be initiated in 2005. Other dissemination actions of relevance to this project are shown below in chronological order.

April 2003 – Oral presentation for university college students and teachers. Title: Beyer, J., ”Biomarkører og effektmål for miljøvurdering innen offshore næringen”. 10. April 2003, Stavanger University College, Stavanger. (Ref.: Prof. Helene Schei, HiS).

September 2003 - Oral presentation at international scientific conference Beyer, J., S. Plisson-Sauné, L. Pinturier-Geiss, L. Chancerell**,** H. Berland, R. Sundt, A. Bjørnstad and S. Sanni “The environmental biomarker survey at Frøy – 2003”. Forum for havmiljøovervåking, Oljeindustriens Landsforening (OLF), 17. september 2003, Quality Airport Hotell – Sola, Stavanger.

September 2003 - Oral presentation at international scientific conference: Beyer, J., Aas, E., Jonsson, G., “PAH metabolites in fish bile for environmental monitoring of produced water discharges at North Sea oil fields: Method optimisations and field data”. ISPAC 19 conference, 22-25 September 2003, Amsterdam, The Netherlands.

October 2003 – Oral presentation at NFR PROFO & PROOF annual meeting: Beyer, J., “Pollutant exposure and effects in fish related to the discharge of produced water in the North Sea oil industry” Hotel Olavsgaard, Gardermoen, 14-15 October 2003. (Ref.: Eli Rinde, NFR).

November 2003 – Oral presentation at public seminar for teachers. Title: Beyer J., "*Biomarkers in fish:* Biological, physiological and histological markers of pollution stress". MNT-forum Sør-Rogaland: Forum for matematikk, naturfag og teknologi (i samarbeid med Statens utdanningskontor og Rogaland fylkeskommune, opplæringsavdelingen). 27 November 2003. Stavanger. (Ref: Kjell Johnsen, MNT-forum / Stavanger offshore tekniske skole).

March 2004 - Oral presentation at international Symposium. Title: Jonny Beyer, Grete Jonsson, Stig Westerlund, Thierry Baussant, Endre Aas, Steinar Sanni, Lennart Balk, Ketil Hylland, Bjørn Munro Jenssen, Marco Mascini, Graziana Bagni & Jarle Klungsøyr (2004). "Towards zero harmful discharges from North Sea oil and gas installations: Results from recent and ongoing effect studies" Proceedings: The 15th Int. Oil Field Chemicals Symposium (OFCS), 28-31 March 2004, Geilo Norway, paper 20, pp 13.

April 2004 - Beyer J., *"Biomarkører og effektmål for miljøvurdering innen offshore næringen."* Oral presentation for students at Stavanger University College 16th April 2004 (ref Helene Skei, Stavanger Univ College).

May 2004 – Oral presentation for industry related participants and research scientists at NFR-PROOF Workshop, arranged May 5th 2004 at Norsk Forskningsråd Oslo. Title: Beyer J. & S. Sanni. *"PROOF midt i løpet – uløste oppgaver og samkjøring innen kjemisk karakterisering."* Ref. F. Fonnum/E. Rinde, PROOF.

May 2004 – Oral presentation of the project results for the oil and gas industry participants at the TOTAL workshop arranged at Sola Strandhotel in Stavanger the 13th May 2004.

October 2004 - Oral presentation at PROOF annual meeting at Bårdshaug Herregård (Orkanger). Title: Grete Jonsson, Admira Cavcic, Tone U. Stokke, Jonny Beyer and Kåre B. Jørgensen (2004). "*Analysis of alkylphenol metabolites in fish bile as a tool for monitoring produced water discharges - Method development and utilisation*".

7 Collaboration and coordination with other projects

This project is coordinated with other ongoing projects that address PW issues. The aim is to achieve add-on value and to avoid parallel activities. The most important of these projects is at present NFR-153692/720 (2003-2005) (Inst. of Marine Research): This IMR project focuses on hormone disruption and possible DNA damage on fish of alkylphenols in produced water from offshore oil installations. The main benefit for present project is collaboration on exposure experiments in 2004 and the exchange of samples.

Appendix documents:

Appendix 1 - WP-1 - Forsøksplan-fellesforsøk 2004

Appendix 2 - WP-2 - Admira Cavcic master thesis

Appendix 3 - WP-2 - Tone Stokke master thesis

Appendix 4 - WP-4 - Work report HRP Biosensor

Appendix 5 - WP-5 - Mitochondrial DNA adducts

Appendix 6 - WP-6 - Preliminary report SBP