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Title of Master's Thesis: **DREAM (Dose-related Risk and Exposure Assessment Model) as a tool to predict biomarker responses in offshore produced water discharge situations**

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DREAM (Dose-related Risk and Exposure Assessment Model) som et verktøy for prediksjon av biomarker responser ved offshore produsert vann utslipp

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Abstract

Every year, approximately 200 million tones of produced water are discharged into the sea by the Norwegian oil industry. Although rapidly diluted, due to its large amount and possible long term (chronic) effects, the environmental risk of produced water discharge has been investigated widely. The risk prediction model called DREAM (Dose-related Risk and Exposure Assessment Model) has been used for environmental risk assessment of produced water discharges. Biological markers or so-called 'biomarkers', have been proposed as a suitable tool for pollutant-effect-monitoring of the discharges from the offshore industry. However, the links between environmental risk model predictions and biomarker responses in produced water exposed animals are still not clearly defined. Therefore, the objective of this study is to investigate the feasibility of linking these two risk tools for the purpose of enabling prediction of environmental risk which can be monitored.

In practice, this is done by employing the DREAM model not only to perform a general risk assessment but also to predict the biomarker responses of produced water discharge and then compare the results with the biomarkers responses measured in a field survey. The link between the model and biomarker response is established using the species sensitivity distribution (SSD) approach.

From the results, it is shown that predicting biomarker responses using the DREAM model was feasible by applying some assumptions and simplifications. The model could also predict a similar trend with the observation responses at different stations. In this case, the predicted biomarker responses give about 14% higher value compared to observation, which is related to the conservative approach (based on the maximum risk value) applied in the model and therefore the model cannot accommodate the duration variable in the biomarker response formation and recovery processes which may differ in biomarkers.

Despite the remaining uncertainties and limitations, especially in relation with the model limitations, reliability of the SSD approach and also the available field data, this study could provide some essential basis for the study of linking the risk prediction with risk monitoring.

Acknowledgement

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I would also like to thank IRIS-Biomiljø for giving me the opportunity to work with this project and to all my supervisors for all your brilliant guidance during my thesis work. I would like to thank Steinar Sanni for introducing me to the Biomarker Bridge concept and guiding me in my work and Emily Lyng for guiding me in the principles of the DREAM model. I also would like to thank Rofl Sundt for giving the guidance in the data processing from the WCM project 2008. My special thanks are dedicated to all people at IRIS-Biomiljø that have helped me during the process from teaching me to use *R* to giving me a ride home.

I also would like to thank ConoccoPhillips for the permission in using the field data of Ekofisk and also to Mathijs Smit for the 'short course' in SSD concept.

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1 Introduction

1.1 Background

Produced water is water that is produced along with oil and gas and that originates from formation and injection water. It is a complex mixture containing hydrocarbons, metals, and potentially toxic production chemicals, (e.g. biocides, corrosion inhibitors, dispersants, emulsion breakers, detergents and scale inhibitors). Every year, approximately 200 million tons of produced water are discharged into the sea from the Norwegian oil industry (OLF, 2008). Although rapidly diluted, due to its large amount and possible long term (chronic) effects, the environmental risk of the produced water discharge has been investigated widely.

To evaluate and estimate the environmental consequences of the discharge, the common environmental risk assessment (ERA) procedure using the PEC/PNEC approach (EC, 2003) combined with the Environmental Impact Factor (EIF) concept (Johnsen et al., 2000) has been adopted to establish the risk prediction model called DREAM (Dose-related Risk and Exposure Assessment Model). The model enables the prediction of concentration fields, biological exposure, doses and potential effects of time-variable exposure to mixtures of chemicals. Therefore the model is much used as a basis for management of environmental risk assessment of produced water discharges.

On the other hand, biological markers or so-called 'biomarkers' have been proposed as a suitable tool for pollutant-effect-monitoring of discharges from the offshore industry. Biomarkers can be defined as measurements carried out in body fluids, cells or tissues that indicate, in biochemical or cellular terms, the presence or effect of contaminants (McCarthy and Shugart, 1990). Since the biomarker responses are measured at the sub-organismal level of organization (biochemical, physiological and histological), they are considered as early warning signals for the presence of contaminants and, thus, suitable for the environmental impact assessment (EIA) purpose.

However, the links between environmental risk model predictions and biomarker responses in produced water exposed animals are still not clearly defined. Therefore, the objective of this study is to investigate the feasibility of linking these two risk tools for the purpose of enabling a prediction of environmental risk which subsequently can be monitored in the field. In practice, it was done by employing the DREAM model not only to perform risk assessment but also to predict the biomarker responses caused by a produced water discharge and then comparing the results with biomarkers responses measured in a field survey.

1.2 Scope of the study

To achieve the objective of this study, the following tasks were included for this master thesis project:

- Develop an understanding of how the DREAM model works for EIF calculations and how PEC/PNEC ratios and species sensitivity distributions (SSDs) are utilized by the model. Corresponding to the available field data, the environmental risk assessment of produced water using DREAM was performed, with Ekofisk field as a study case.
- Use biomarker responses results obtained from laboratory studies in IRIS-Biomiljø in order to create simulations of predicted biomarker responses at different distances from the platform.
- Comparison of predicted biomarker response results with actual biomarker responses obtained from the Water Column Monitoring surveys conducted at Ekofisk (2008).
- Assessment of the use of the near-field module in DREAM for biomarker response predictions.

1.3 Report Outline

The next chapter of this report is dedicated to the description of theories relevant to the background of this study including some concepts in ecotoxicology, description of the DREAM (Dose-related Risk and Exposure Assessment Model) and overview of several types of biomarkers. The methodology involving the simulation of Environmental Risk Assessment (ERA) in DREAM and biomarker responses prediction is described in chapter 3. The results are presented in Chapter 4 and discussed in the following chapter with the conclusions are shown in chapter 6.

2 Theoretical Background

2.1 Basic Concepts in Ecotoxicology

The term ecotoxicology was first introduced by Prof R. Truhaut in 1969, who defined it as a science describing the toxic effect of various compounds on living organisms, especially on population and communities within ecosystems (Connell et al., 1999).

In this study, it is important to have some basic understanding in ecotoxicology that will be extensively used in the following part of this report. Some concepts in ecotoxicology such as bioconcentration or body burden, dose-response relationship, toxicity testing, species sensitivity distribution and environmental risk assessment (ERA) will be discussed in this sub-chapter.

2.1.1 Body Burden

When a chemical enters a marine ecosystem, not all of this pollutant could enter the organism and eventually cause some harmful effects, it may be partitioned between different phases (water, sediment or biota, etc). The internal exposure concentration in an organism is called *body burden*. Body burden is determined by uptake and elimination processes of chemical in an organism which are influenced by several factors such as temperature, ventilation rates, metabolism, type of species and also the characteristic of the chemical (Baussant et al., 2001). For risk assessment, it is important to estimate the body burden that may elicit a toxic response (Feijtel et al., 1997). Body burden usually is expressed as bioconcentration factors (BCF) that is the ratio of substance concentration in the organism to the concentration in the water at equilibrium condition (at which the competing rates of uptake and elimination are equal).

At equilibrium condition, the bioconcentration factor is calculated based on:

$$BCF = \frac{C_b}{C_w} \qquad \text{(eq. 2.1)}$$

where C_b is the concentration in biota and C_w is concentration in water. These bioconcentration factors are specific for each species and compound. The bioconcentration factors of poly-aromatic hydrocarbons (PAH) compounds that were calculated based on lipid weight of different samples of (*Mytilus edulis*) blue mussel and cod (*Scopthalamus maximus*) (Baussant et al., 2001) can be found in Table A-1 (Appendix A).

2.1.2 Toxicity Test

Toxicity tests study the responses of individual organisms or groups of organisms to chemical exposure. The test is typically performed on a population exposed to different concentrations of a chemical under controlled conditions over a specific period of time.

In the toxicity test, the adverse effects of chemicals on the organism depend on the dose and time of exposure. Tests that are based on lethality or survival and designed to evaluate short-term exposure (usually 24, 48 or 96 hours) are called *acute toxicity test*. The acute effects can be quantified by $\mathbf{LC_{50}}$ (the concentration that cause 50% mortality of the test organisms) or $\mathbf{EC_{50}}$ (the concentration at which 50% of the predicted effect is observed). On the other hand, the *chronic toxicity tests* that allow evaluation of chemical stress under long term exposure at sub-lethal concentrations are commonly quantified by **NOEC** (No-Observable Effect Concentration) and **LOEC** (Lowest Observable Effect Concentration).

The results of the tests can be plotted on a graph that relates the chemical concentration to the percentage of organisms in test groups exhibiting a defined response, such a is relationship is called a *concentration-response relationship* (see Figure 2-1).

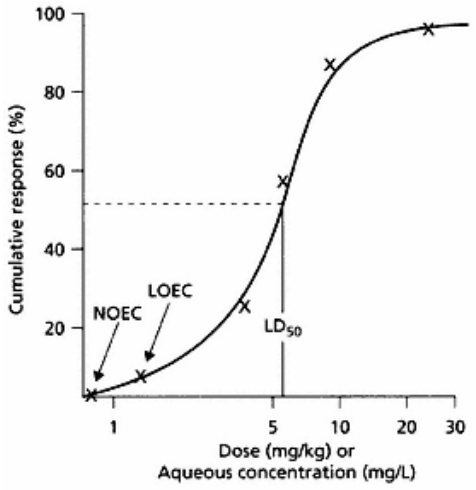


Figure 2-1. Cummulative dose response curve with LOEC, NOEC and LD50 are indicated. (Connell et al., 1999)

To prevent the multiplication of the toxicity test, the internationally accepted standard testing protocols are organized by OECD (Organization for Economic Cooperation and Development). Some tests standardized by OECD i.e. growth inhibition test of algae, acute toxicity test of zooplankton and acute toxicity test of fish are mandatory tests for toxicity testing of offshore chemicals in Harmonized Offshore Chemical Notification Format or HOCNF (OSPAR, 2008). Due to the shorter time needed and therefore the lower cost involved, single-species acute toxicity tests have become the largest part of the toxicity studies. To estimate a safe or chronic concentration from acute tests, the acute-to-chronic-ratio (ACR) has been evaluated (Wright and Welbourn, 2002).

In addition to the acute toxicity tests, the bioaccumulation potential and biodegradation rate of a substance are also included guidelines in the HOCN information on chemicals discharged from offshore installations (EC, 2003; OSPAR, 2008).

2.1.3 Species Sensitivity Distribution (SSD)

Toxicity responses of different species vary due to biological differences. The variation in sensitivity of species to a certain compound or mixture, described by a statistical or empirical distribution of response is called *species sensitivity distribution* (SSD). The main assumption in the use of SSDs in risk assessment is that the distribution based on a selection of species (tested in laboratory experiments) is representative for all species in the ecosystem.

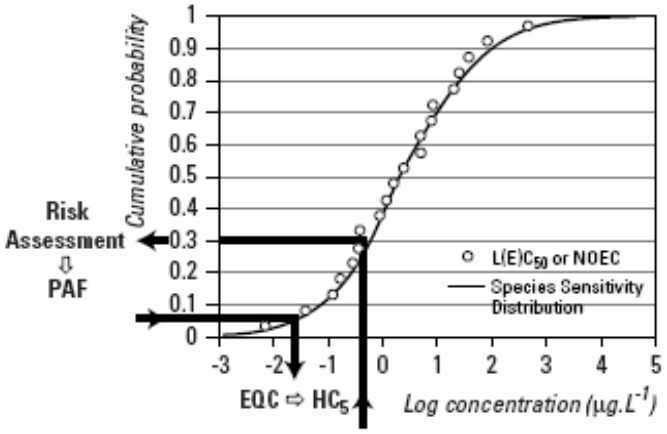


Figure 2-2. The basic form of an SSD curve, expressed as cumulative distribution function. The dots are input data from toxicity tests and the line is a fitted SSD (Posthuma et al., 2002).

The SSD can be presented as a frequency distribution (cumulative normal distribution curve or other similar curves) of NOECs (No-Observable Effect Concentrations) or other results from toxicological tests as explained by Posthuma (2002) and Aldenberg (2002). Toxicity data (NOEC, EC50, etc) are log transformed and fitted to a distribution function (Figure 2-2).

Figure 2 - 2 also shows the two ways of utilizing the SSD curve: *forward* and *inverse*. In the forward way, the distribution can be used to estimate risk at a specific concentration that is expressed by *potentially affected fraction* (PAF) i.e. the percentage of species that are exposed to concentrations above their NOEC. PAF can be used to represent the stress to the ecosystem caused by a single chemical, or to map the total stress on the ecosystem as a result of the concentration of several chemicals or chemical groups. The inverse usage of the model employs the distribution for calculating environmental quality criterion for a certain cut-off value, e.g. the 5th percentile or HC_5 (i.e. the concentration that corresponds to 5% risk). The 5th percentile of a chronic toxicity distribution has often been chosen as the concentration which is considered protective for most species in a biological community.

2.1.4 Environmental Risk Assessment (ERA)

Environmental Risk Assessment (ERA) evaluates the possible occurrence of adverse ecological effects of pollutants in a manner as quantitative as possible. For this purpose, the main procedures of ERA consist of 4 main steps as seen in Figure 2-3 (EC, 2003; van der Oost et al., 2003; Wright and Welbourn, 2002):

- hazard identification,
- exposure characterization
- effect characterization,
- risk characterization.

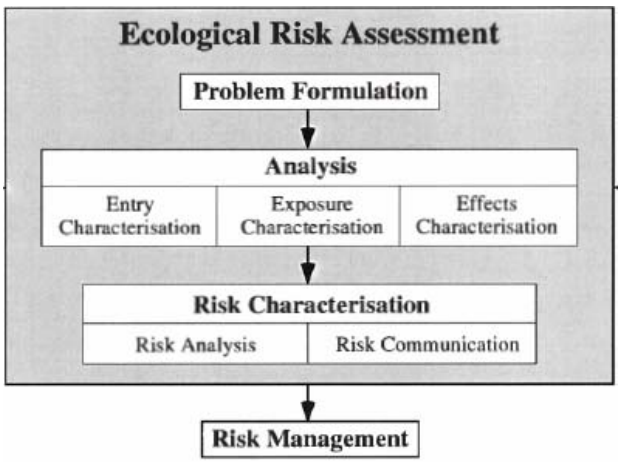


Figure 2-3. General Environmental Risk Assessment scheme (Wright and Welbourn, 2002).

Hazard identification is a qualitative step, either based on former knowledge of the substance or on the fact that no knowledge exists, therefore applying precautionary principles on a new substance.

The exposure characterization involves the method to quantify the concentration of a discharged chemical in the environment to obtain the PEC (Predicted Environmental Concentration). The PEC value can be calculated through measurement and also from modeling of chemical fates in the environment.

Effect characterization is a process to predict the adverse effect of chemicals in biological recipients that is represented by a Predicted No Effect Concentration (PNEC) which indicates a concentration, below which, an unacceptable effect will most likely not occur. When only a limited set of toxicity data is available, PNEC is calculated by dividing the laboratory effect concentrations (LC_{50}, EC_{50}, NOEC, etc) by appropriate *assessment factor*s. Some example of assessment factors for marine ecosystem can be seen in Table 2-1. When sufficient data is available, PNEC value may also be derived from SSD based on chronic NOECs by taking the 5^{th} percentile of the distribution (i.e. the concentration that corresponds to 5% risk) (Aldenberg and Slob, 1993)

Table 2-1. Assessment factor scheme as used for calculating PNEC values (EC, 2003).

Available toxicity data	**Assessment factors**
At least one short term EC50 from each of three trophic levels (algae, crustaceans and fish)	1000
Long term NOEC representing two trophic levels (fish and/or crustacean and/or algae)	100
Long term NOEC from at least three thropic level (fish, crustaceans and algae)	10

The next step is to compare the predicted environmental concentration (PEC) with the threshold concentration (PNEC) and present it in the form of PEC/PNEC ratio or Risk Characterization Ratio (RCR). This ratio will be used as a basis to evaluate the potential risk. An RCR that exceeds 1.0 indicates that there is reason for concern (i.e. an effect is foreseen) and thus some risk reduction measures are needed.

2.2 DREAM (Dose-related Risk and Exposure Assessment Model)

2.2.1 Introduction

DREAM is a software tool designed to meet the need of rational basis in management of environmental risk assessment associated with operational discharges of complex mixtures. It has been developed in cooperation of several research centers (Akvaplan-niva, Battelle, MUST, IRIS-Akvamiljø, SINTEF, TNO and the University of Oslo) and petroleum companies operating in the Norwegian continental shelf (ConocoPhillips, Eni, ExxonMobil, StatoilHydro, Petrobras, Shell, and Total).

Another model called a 'chemical hazard assessment and risk and management' (CHARM) model has also been much used. The CHARM model enables ranking of chemicals based on their properties and uses a fixed dilution factor, assuming equal and constant dispersion (Karman and Reerink, 1998). However, in reality, the chemical fates follow the three-dimensional dispersion that change over time. In order to provide more realistic dynamic risk assessment, DREAM (Dose-related Risk and Exposure Assessment Model) was developed.

Environmental Risk Assessment with DREAM utilizes the Environmental Impact Factor (EIF) concept which is based on PEC/PNEC approach as described by the European Union in a Technical Guideline Document (EU-TGD) (EC, 2003). However, DREAM-EIF applies some modifications from the EU-TGD method that accounts for the complex mixture of chemicals in produced water and the differences in their fates and toxicities in the marine environment (Johnsen et al., 2000).

2.2.2 Physical-chemical fate modeling

Calculation of the environmental concentration (PEC) is the basis for risk assessment. In DREAM, PEC is calculated by modeling the fates of pollutants in the environment. The fate module of DREAM is a dynamic three-dimensional, multiple-component pollutant transport model.

Governing equation

The fate model is based on the general transport equation (Reed et al.):

$$\frac{\partial}{\partial t}C_i + \vec{V}\cdot\vec{\nabla}C_i = \vec{\nabla}\cdot D_k\vec{\nabla}C_i + \sum_{j=1}^{n} r_j C_i + \sum_{j=1}^{n}\sum_{j=1}^{n} r_{ij} C_i \qquad \text{Eq. 2.2}$$

where C_i is the concentration of the ith chemical constituent in the release, t is time, $\boldsymbol{V}$ is advective transport vector, $\vec{\nabla}$ is the gradient operator and D_k is the turbulent dispersion coefficient in k=x,y,z direction. The term $\mathbf{r}_j$ are process rate including:

- addition of mass from continuous release
- evaporation from surface slicks
- spreading of surface slicks
- emulsification of surface slicks
- deposition from water surface onto coastline
- entrainment and dissolution into water column
- resurfacing of entrained oil
- volatilization from water column
- deposition from water column to bottom sediment, etc.

Meanwhile, the term $\mathbf{r}_{ij}$ represents the degradation process in the model.

The chemical concentration, C_i in the water column is calculated based on the time-and space-variable distribution of pseudo-Lagrangian particles. There are two types of particles, those representing dissolved substances and those representing oil droplets or particles with non-neutral buoyancy. The latter particles are pseudo-Lagrangian in the way that they do not move strictly with the current but may rise or settle according to their buoyancy (Reed et al.).

Physical-chemical Fate Processes

When pollutants enter the marine environment, they will go through physical-chemical processes, such as advection, dispersion, volatilization, dissolution and degradation. The processes governing the pollutants fates in DREAM are described in Figure 2-4.

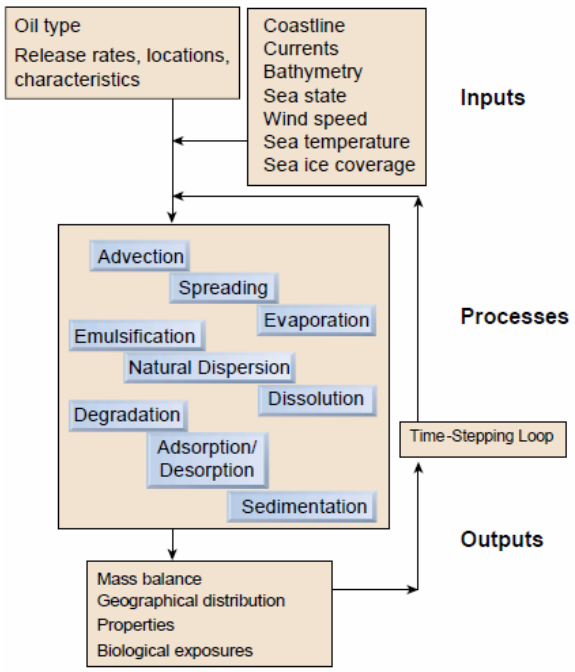


Figure 2-4. General layout of the DREAM model (Reed et al.)

Advection and *dispersion* of the entrained and dissolved hydrocarbons in the water column, are controlled by the mean local velocity as a result of tidal, wind-driven and wave-driven components.

Pollutants near the water surface may *evaporate* to the atmosphere. The rate of mass transfer from the water column to atmosphere is calculated using the procedure outlined by Lyman et al. (1982).

Adsorption is important in the transport and fate of pollutants in the marine environment since it determines the extent of partitioning of a pollutant between the suspended particulate phase and the dissolved phase and, therefore, governs toxic effects as well as the rate of removal from water column to the sediments. The partitioning between the particulate-adsorbed and dissolved states is calculated based on the linear equilibrium theory. The contaminant fraction that is adsorbed to suspended particulates settles to the bottom.

The DREAM model also takes into account the transformation of components via *degradation* transformation pathways since it is known that the degradation products may be more soluble and toxic than the parent compounds.

Physical environments included in DREAM

The physical environment in DREAM is defined by several parameters including:

- Bathymetry (depths)
- Wind and wave fields
- Currents
- Sea temperature, salinity.

Bathymetry (depth) of the selected location is defined by a gridded dataset, stored in a database. The standardized winds, wave and current fields are provided in the database as separated input files, but it is possible for the user to utilize different files. Alternative current, wind and wave fields can be utilized by importing selected format of file or by defining them through the user interface of DREAM. The vertical profile of temperature and salinity is added by the user to calculate the water density of the region.

2.2.3 Environmental Risk Assessment using DREAM

Risk assessment with DREAM uses the EIF (Environmental Impact Factor) concept. It is basically follows the PEC/PNEC approach which is comparing the predicted exposure concentration (PEC) and predicted environmental toxicity threshold (PNEC). However it also applies weighing factors to account for the persistence of chemicals and their tendency to bioaccumulate. The complete scheme of risk assessment in DREAM is presented in Figure 2-5.

The environmental concentration (PEC) is calculated by fates modeling as described in section 2.2.2. The calculation is done by dividing the produced water into several chemical groups and each group represents a class of chemical with similar physical, chemical, toxicological and biological properties. Produced water chemicals were divided into 10 groups of naturally occurring compounds and 7 groups of additives (Johnsen et al., 2000). Additional chemicals specific to each release are user defined

according to the HOCNF data. These chemical groups are listed in Table B-1, Appendix B. A more detailed list of the compounds included in each group can be found in Table B-2, Appendix B.

Although widely used in risk assessment, the PEC/PNEC approach doesn't give the actual risk level. It only gives the indication whether the pollutant concentration in the ecosystem has exceeded the threshold level or not and, therefore, the PEC/PNEC approach is only a qualitative indication of potential risk level. To translate the PEC/PNEC into actual risk level, species sensitivity distribution is used (Karman and Reerink, 1998; Smit et al., 2005). When this distribution is based on long term NOECs, PNEC corresponds with the 5^{th} percentile of this distribution. When insufficient, the same distribution can be estimated from the PNEC (that is obtained by applying the assessment factors) and an indication of the variation in sensitivity of species for this chemical (presented by the slope of the curve). Using this distribution, a corresponding risk value can be calculated at any given exposure concentration (refer to section 2.1.2 about species sensitivity distribution [SSD]). The same method is applied for all components and combined to calculate the total risk representing the produced water discharge.

The EIF concept also applies weighting to certain compounds based on biodegradability and bioaccumulation potential (see Table B-1, B-3 and B-4, Appendix B). The EIF is presented as total water volume for which **PEC/PNEC** ratio exceeds **1.0** with maximum resolution 100mx100mx10m (100,000 m^3 volume).

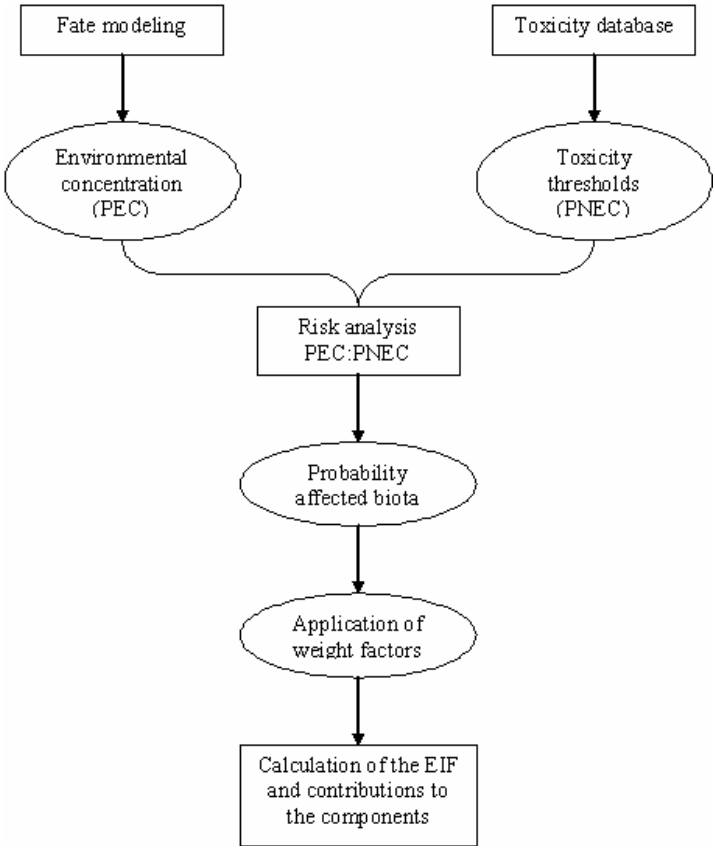


Figure 2-5. Process scheme of the calculation of EIF for produced water discharge (Smit et al., 2003)

2.2.4 User defined parameters in DREAM calculations

Substances setting

The data of different groups of chemicals are combined in the *release profile*. Each substance is registered with the following characteristics: viscosity, mol weight, density, melting and boiling points, solubility, vapour pressure, octanol-water partition coefficient, and degradation rates. For the purpose of risk assessment, acute and chronic toxicity sensitivities for different species are also included. For the naturally occurring components, all the data are already available in the database, while for the added chemicals, these data can be obtained from the HOCNF (Harmonised Offshore Chemical Notification Format) document.

Scenario parameters

To set up a new release scenario, some information such as location, depth and amount of the produced water release need to be set accordingly. The duration and start time for the simulation are also adjusted here. The physical environments such as current, wind, temperature and salinity of the ocean are set according to the release location. DREAM also facilitates multiple discharge points and long term simulations (stochastic).

Model setting

The model parameters affect accuracy, resolution in space and time, size of output files and computational speed. Therefore model parameters need to be set accordingly in order to have optimum simulation results.

2.2.5 Simulation outputs

The output from DREAM includes the concentration field of the pollutants, risk map for the modeled area, EIF value giving the recipient water volume which RCR (PEC/PNEC ratio) ≥ 1.0 and also a pie chart showing the contribution to risk from different groups of chemicals. In addition to graphical presentation, the value of environmental concentration and risk can be extracted from the model in text-file format. An example of the risk map is presented in Figure 2-6.

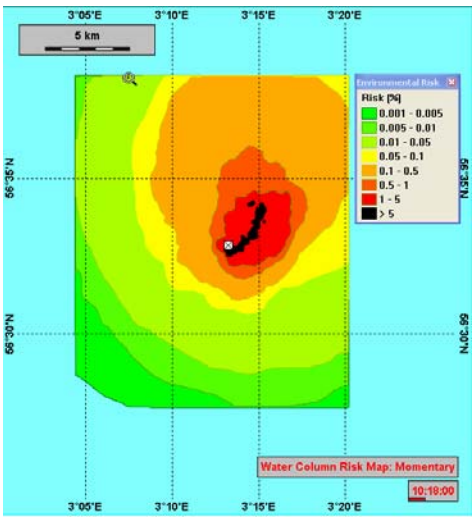


Figure 2-6. Example of graphical output in DREAM

2.3 Biomarkers

2.3.1 Introduction

The marine environment is continuously loaded with foreign chemicals (xenobiotics) that are discharged directly or that come from land sources and via the atmosphere. The ability of pollutants to accumulate, transform and degrade complicates the study of pollutant exposure to marine ecosystem. The harmful effects on population become apparent after longer periods of exposure. When they finally become clear, they may have gone beyond the point where it can be reversed. Therefore, it is important to study the biological markers that could reflect the early responses to adverse pollutant stress, or early-warning signals (van der Oost et al., 2003).

Biological markers or biomarkers can be defined as any measurement in body fluids, cells or tissues that indicate, in biochemical or cellular terms, the presence of contaminants or the magnitude of the response (McCarthy and Shugart, 1990). In a biomonitoring context, biomarkers can allow rapid assessment of organism health and also they are quantifiable biochemical, physiological or histological measures that relate in a dose-response or time-dependant manner the degree of dysfunction that the pollutant has produced (Mayer et al., 1992)

2.3.2 Types of Biomarkers

The responses of biomarkers can be considered as *exposure* or *effect* indicators. Biomarkers of exposure can be used to confirm and assess the exposure of species to a particular substance and thus providing the relationship between external exposure and internal dose. Biomarkers of effect include measurable biochemical, physiological or other alterations within tissues or body fluids of an organism that can be associated to external exposure of a chemical. PAH metabolites in bile is an example of a biomarker of exposure. DNA damage and lysosomal membrane stability alteration can be regarded as biomarkers of effect, although they can also serve as indicators of exposure.

2.3.3 Selection of Biomarker

In selecting the appropriate and useful biomarkers for monitoring the pollutant exposure and effects, there are several criteria to be considered (Stegeman et al., 1992):

1. The assay to quantify the biomarker should be reliable, relatively cheap and easy to perform;
2. The biomarker response should be sensitive to pollutant and/or effects in order to serve as an early warning system;
3. Baseline data of the biomarker should be well defined in order to distinguish between natural variability (noise) and contaminant induced stress (signal);
4. The impacts of confounding factors to the biomarker response should be well established;
5. The underlying mechanism of the relationship between biomarker response and pollutant exposure (dosage and time) should be well established;

The toxicological significance of the biomarker, e.g. the relationship between its response and the (long term) impact to the organism should be established

2.3.4 Biomarkers used in marine environmental risk assessment

The concern about possible long term ecological impact from chemical contamination from offshore activities in the North Sea has introduced the need for monitoring tools capable of detecting subtle biological responses of exposed populations. Biomarkers have been proposed as suitable pollutant-effect monitoring tools for the offshore industry. For this purpose, various biomarker responses in produced water exposed fish and marine invertebrates have been investigated by IRIS-Biomiljø. Several biomarkers that have been studied and made available for this study by IRIS-Biomiljø include PAH metabolites as an exposure biomarker, DNA adduct formation as a biomarker of genotoxic pollutants and lysosomal membrane stability as nonspecific defence parameter.

2.3.4.1 PAH Metabolites Biomarker

The exposure to certain common environmental contaminants such as poly-aromatic hydrocarbons (PAHs) usually cannot be assessed by direct analysis for these chemicals, because they are rapidly converted to a variety of metabolites (McCarthy and Shugart, 1990; Melancon et al., 1992). The metabolites may accumulate to high levels in certain tissues or body fluids or bind to specific tissue macromolecules in a manner that facilitates detection of exposure and indicates potential harmful effects.

In fish, detection of PAH metabolites in bile has been shown to be an excellent tool in assessing recent exposure to PAHs. The determination of PAH metabolites has been proposed as a biomarker of PAH exposure by international bodies such as OSPAR (Oslo-Paris Commission) and ICES (International Council for the Exploration of the Sea) (Hagger et al., 2006; ICES, 2004).

Metabolite levels in bile can be determined either by quantitative assay of selected PAH metabolite or by analyzing the total level of PAH metabolites as fluorescent aromatic compound (FAC) (See Figure 2-7). The quantitative assay of selected PAH metabolites can be done using HPLC (High Perfomance Liquid Chromatography) or Gas chromatography/mass chromatography (GC/MS), meanwhile, semi-quantitative assays can be performed using synchronous fluorescence spectrometry (SFS), fixed wavelength fluorescence (FF) or HPLC (Beyer and Bamber, 2004).

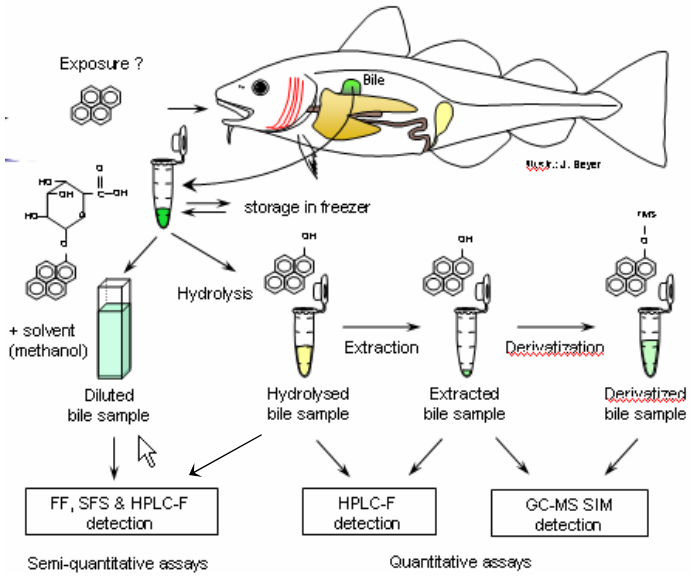


Figure 2-7. Overview of method alternatives for detection of PAH metabolites in fish bile using pyrene as an example contaminant (Illustration by Jonny Beyer)

2.3.4.2 DNA Damage

Many pollutants investigated have shown to be chemical carcinogens and mutagens with the capacity to cause various types of DNA damage. The interaction of toxicants with DNA is demonstrated primarily by structural alterations to the DNA molecule and can take the form of adducts, strand breakage or chemically altered bases. These lesions may raise irreversible changes to the DNA molecule and result in the expression of subsequent cellular responses such as chromosomal aberrations and *oncogene* activation. The detection and quantification of DNA alteration and subsequent effects may be employed as biomarkers in organisms exposed to *genotoxic* substances in the environment.

DNA adducts

A DNA adduct is formed when a non-DNA chemical, e.g. carcinogenic chemical or its metabolite, binds covalently to DNA. As an example, a model of DNA adduct formation of benzo[a]pyrene is described in Figure 2-8. In fish, DNA adducts are most often measured in the liver since it is the key organ for biotransformation of xenobiotics, though other tissues can also be used for this analysis. DNA adducts can also be formed in invertebrates following exposure to pollutants, but this occurs at much lower intensity than in fish.

Detecting and quantifying DNA adducts are not simple tasks because analytical techniques currently available are limited in their sensitivity or specificity. The most sensitive assay available for measuring DNA adducts is 32P-postlabeling, but other methods e.g. HPLC/fluorescence spectrometry and immunoassays using adduct-specific antibodies are also available.

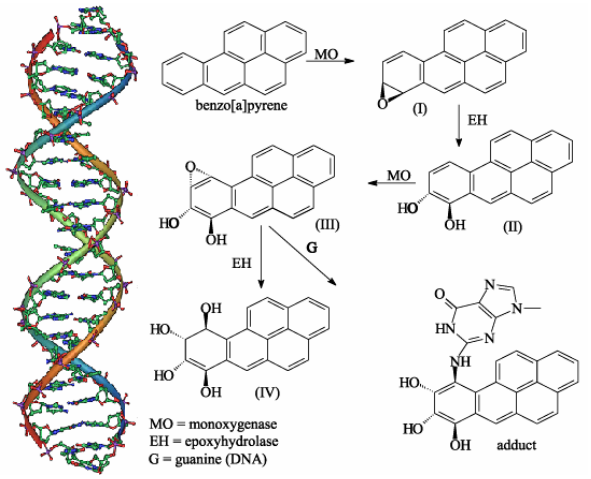


Figure 2-8. Model of DNA with addcuted molecule of benzo[a]pyrene diol-epoxide

DNA strand breakage

Beside direct adduct formation, damage due to carcinogenic pollutant exposure also include DNA strand breaks. Several methods including the alkaline unwinding assay and the comet assay can be used to investigate the strand breaks level in organisms exposed to pollutants.

The alkaline unwinding technique takes advantage of the characteristic that DNA strand separation under defined conditions of pH and temperature occurs at sites of single-strand breaks within the DNA molecule. The amount of double stranded DNA remaining after a given period of alkaline unwinding is inversely proportional to the number of strand breaks present at the initiation of the alkaline exposure.

The comet assay is based on the detection of DNA fragments from single cells which, when following *electrophoresis* under alkaline conditions migrate away from the nuclear core, resulting in the formation of a comet like 'tail' when the cell preparation is stained and viewed under UV light. The length of the tail is a measure of the number of small DNA fragments and thus the amount of strand breaks present in the sample.

Micronuclei

Micronuclei are chromosomal fragments or whole chromosomes that are not incorporated into daughter nuclei during mitosis. The micronucleous test detects micronuclei resulting from either chromosomal breakages during cell division or chromosome loss events in anaphase damages (Kirsch-Volders et al., 2003). The micronuclei assay has been shown to be a useful in vivo technique for genotoxicity testing in fish, invertebrates and marine mammals (Al-Sabti and Metcalfe, 1995; Gauthier et al., 1999; Hongell, 1996).

2.3.4.3 Lysosomal Membrane Stability

Lysosomal membrane stability is considered to be a general measure of stress (both chemical and other) (Moore, 1985). Theoretically, membrane stability decreases in response to stress as membrane permeability increases. The mechanism of this alteration in membrane stability may involve direct effects of chemicals or the increased frequency of secondary lysosomes in toxicant-stressed cells (Mayer et al., 1989).

Lysosomal membrane stability in macrophages (or white blood cells) is used as a measure of pollutant stress in several species of invertebrates such as blue mussels, whelks, hermit crabs and sea stars. It is also possible to carry out analysis on samples taken from fish. A large number of pollutant effect studies using invertebrates have included this parameter as a biomarker. It has been shown to be responsive to major classes of environmental pollutants including heavy metals (in particular Cu), PAHs, HCHs, PCBs and biocides such as TBT

The lysosomal stability condition is measured by means of the so-called Neutral Red Retention Time (NRRT) assay. The assay basically quantifies the retention time of red dye by the lysosomes of contaminant exposed mussels (Lowe and Pipe, 1994; Lowe et al., 1995).

3 Methodology

3.1 Concept

The motivation for this study is to establish *links* between environmental risk model predictions of offshore discharges and the biological effect of produced water contamination by using the DREAM model not only to perform risk assessment but also to predict biomarker responses. The link is established by applying the species sensitivity distribution (SSD) approach. As explained before, DREAM applies SSDs based on toxicity tests to predict the possible risk (EIF) of produced water discharge. SSDs based on biomarker responses called Biomarker Response Distribution (BRD) are then used to simulate the possible effects measured by means of biomarker assays.

In general, there are four steps involved in order to establish the links between environmental risk model prediction and biomarker responses. The first step is to perform risk assessment of produced water using DREAM. Then, the SSD based on biomarker response is built using biomarker data in produced water exposed fish and mussels, made available by IRIS-Biomiljø. This SSD based on individual biomarker is applied to the model in order to predict the biomarker responses at different distances from produced water discharge point. As a validation, the predicted biomarker responses are compared to the biomarker data obtained from a field survey at Ekofisk. These processes are illustrated in Figure 3-1.

In this project, the Ekofisk field is chosen as the study area due to the coherency with the field measurement of biomarker responses available. This field data of biomarker responses are obtained from the Water Column Monitoring Project (IRIS-Biomiljø) and kindly made available for this master thesis by the project clients (ConoccoPhillips).

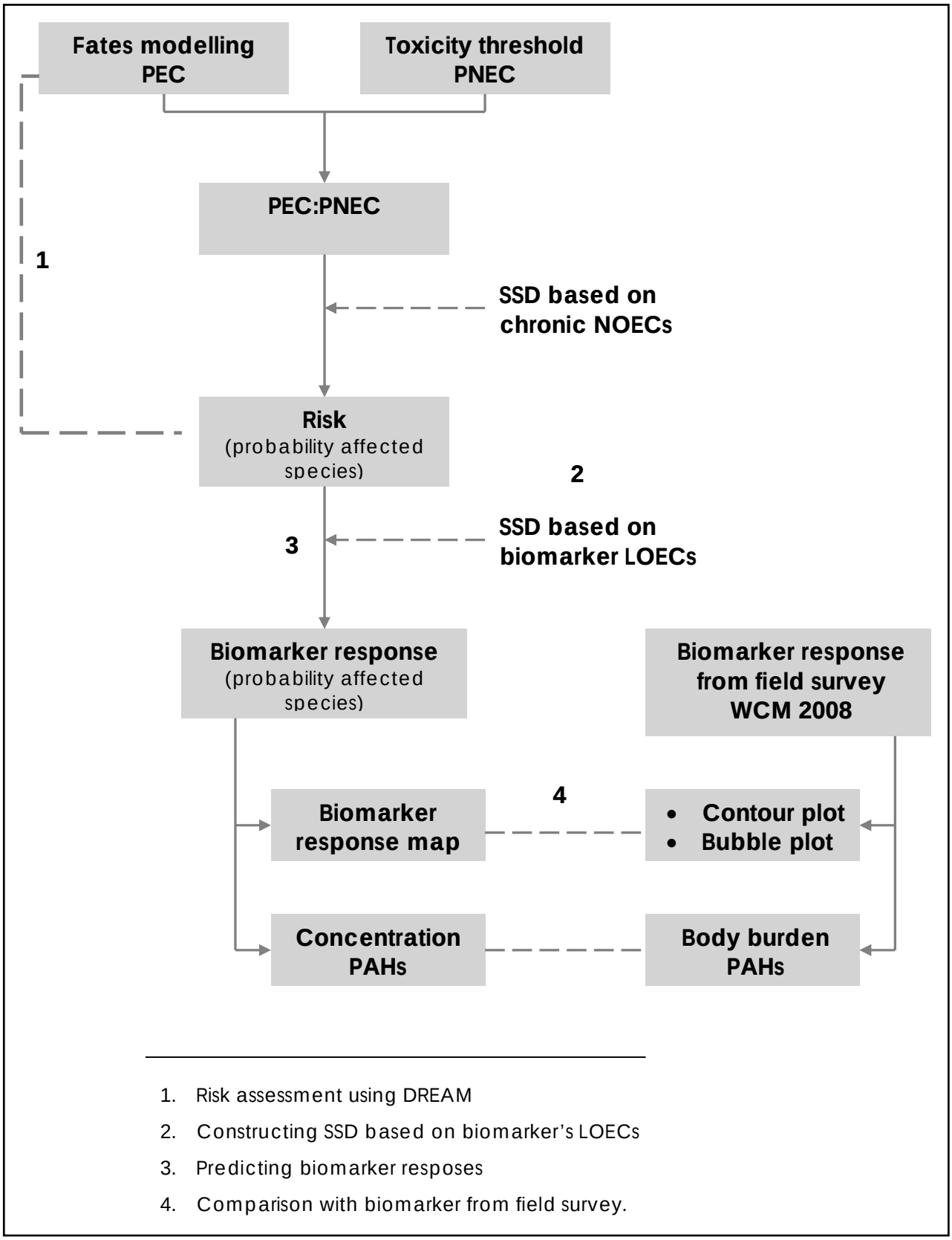


Figure 3-1. The complete scheme of the methodologies used in predicting biomarker responses using DREAM.

3.2 Ekofisk Field

Ekofisk is the oldest field complex in operation on the Norwegian continental shelf started its production in 1971 (Figure 3-2). The sea depth in the area is about 70-75m meters. The field is owned by several oil companies including ConoccoPhillips who are also the operator. Ekofisk consists of several platforms, but only Ekofisk J will be considered in the simulation since it is the main processing facility and has the largest contribution to the total produced water discharge.

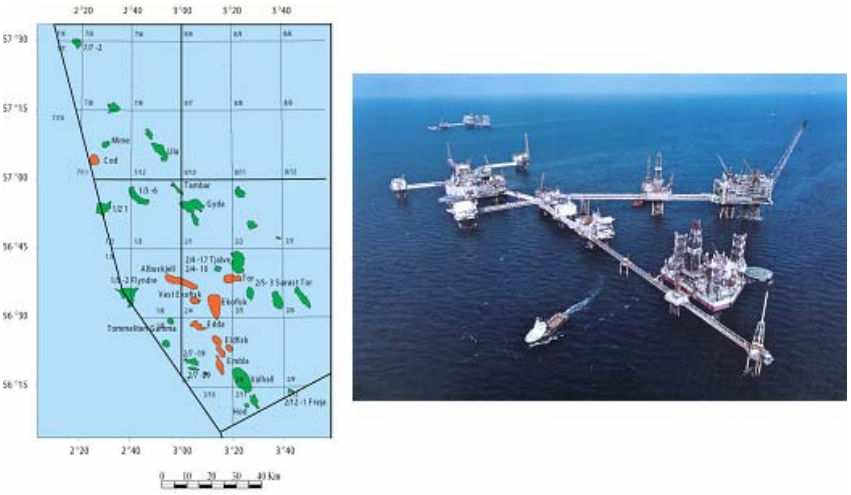


Figure 3-2. Map of Ekofisk region (source: www.npd.no)

3.3 Risk Assessment of Produced Water using DREAM

3.3.1 Data input and scenario set up

To perform environmental risk assessment of produced water discharge using DREAM, there are two types of information used as input: produced water discharge data and the physical environment data. The discharge information includes the amount, location, depth and also the concentration of different chemical compounds in the discharge. Meanwhile, the physical environment data that are already incorporated in the DREAM database include depth, wind and ocean current.

The discharge information for Ekofisk region used in this study are available from ConocoPhillips. The toxicological properties needed for risk calculation (e.g. biodegradation, $\log P_{ow}$ and PNEC) of natural occurring compounds are standardized in the model database, while data for added chemicals are obtained from HOCNF. The overview of the discharge information is presented in Table 3-1.

Table 3-1. Overview of the discharge information from Ekofisk field includes the location, depth, amount and concentration of the naturally occurring compounds.

Position	3° 13.26' E 56° 32.8' N
Depth	39 m
Discharge amount	23 688 tones/day
Natural occurring compound	**Concentration (mg/l)**
BTEX	10.8000
Napthalenes	1.3063
PAH 2-3 ring	0.2791
PAH 4 ring+	0.0066
Phenols C0-C3	6.9560
Phenols C4-C5	0.0204
Phenols C6-C9	0.0030
Aliphatic hydrocarbons	20.0000
Zinc	0.0227
Copper	0.0042
Nickel	0.0069
Lead	0.0012
Cadmium	0.0001
Mercury	0.0005

Standardized current fields and wind time series are chosen based on the region of the release site. The standardized modeling period is 1.5 – 30.5.1990 (30 days). For Ekofisk, the current and wind files from North Sea region are selected;

Current : May90.DIR

Wind : Ekofisk.wnd

To enable the use of the *near field* profile in this simulation, the temperature and salinity profile of the region is set based on the data CTD measurement from the Water Column Monitoring (presented in Figure C-1(b), Appendix C). It is assumed that the salinity and temperature of the water mass don't change so much in time and also the horizontal variation of salinity and temperature can be ignored.

All release information and physical environment data are set in the *scenario parameters* while the produced water chemicals are combined in *release profile* (see Figure 3-3)**.**

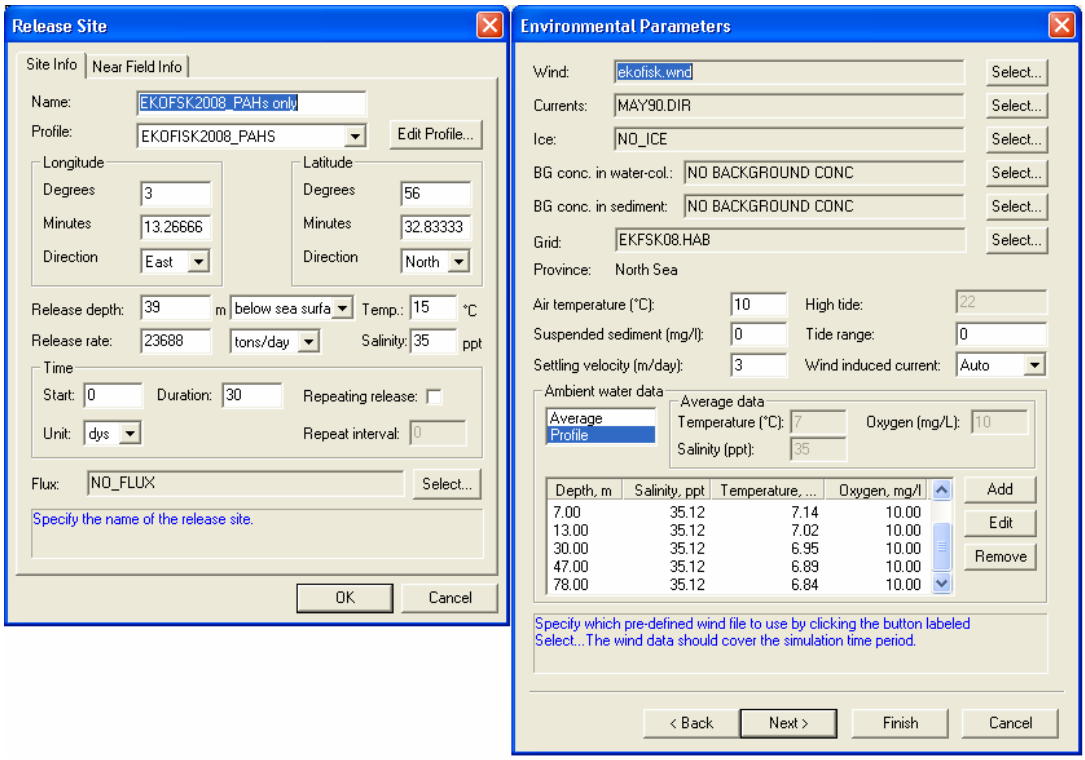


Figure 3-3. Overview of windows for setting-up release scenario and environmental parameters

3.3.2 Model Set up

To have an optimum computation process, there are several model parameters that have to be adjusted. These parameters include:

- *Habitat grid* is the domain in which the model operates. This has to be defined before starting the simulation.
- *Concentration grid* defines grid sizes at which the model computes and reports concentration in the water column.
- *Time step* specifies the time interval between subsequent calculations. Smaller time steps are required when rates of change are more rapid.
- *Number of particles* influences the statistical stability of the results. It is suggested that 1000 active particles will produce stable results for 100mx100m concentration grid and 5-minute time step (OLF, 2003).
- *Lower concentration limit:* the lowest concentration that will be recorded in the output files. The lower concentration limit is set to be 10% of the lowest PNEC

- *Output interval* determines at which frequency the concentration fields and risk results are written to the output files.

The values for these model parameters used in this simulation are summarized in Table 3-2. These values are adjusted on model parameters as presented in Figure 3-4.

Table 3-2. Some model parameters used in the simulation

Model parameters	Values
Size of habitat grid	20km x 20km x 100m
Concentration grid	200 x 200 x 10 cells
Gird resolution	100m x 100m x 10m
Time step	5 minutes
Number of solid and liquid particles	1000
Number of dissolved particles	1000
Lower concentration limit	0.001 ppb
Output interval	6 hours

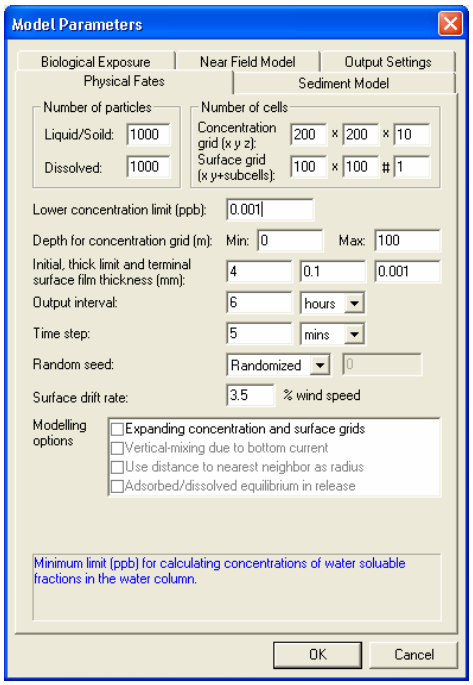


Figure 3-4. Overview of windows for model parameters set-up

3.4 Constructing SSD Based on Biomarker Responses

3.4.1 Biomarker data

To build a SSD based on biomarker responses, biomarker measurements from different marine organisms (fish, crustacean, mollusk and echinoderm) exposed to dispersed oil obtained from laboratory studies at IRIS-Biomiljø are used. Dispersed oil is used as an approximation to produced water. The biomarkers selected for this purpose are PAH metabolites, DNA damage, and lysosomal membrane stability. The available biomarkers for DNA damage include DNA adducts and DNA strand breaks (measured with alkaline unwinding and comet assay). For lysosomal membrane stability, the data of Neutral Red Retention Time (NRRT) are available in the database.

Due to the larger dataset available, the lowest oil concentration that gives significant biomarker response from controls or lowest observed effect concentrations (LOECs) are selected, instead of NOECs. Since it will be used as monitoring parameter, it seems more convenient to use the lowest concentration where the responses are actually measured rather than the highest concentration where the responses are not measured. If a species was tested by more than one type of oil, resulting in more than one LOEC data per species, the geometric mean value was taken to represent the LOEC (Slooff, 1992). These data are presented in Table 3-3.

Table 3-3. Overview of lowest observed effect concentration (LOECs) for biomarkers as total hydrocarbon concentration indicating PAH metabolites in bile, DNA damage, oxidative stress and lysosomal membrane stability in different marine organisms exposed to dispersed oil from IRIS-Biomiljø.

Species	Group	No. of LOECs	Biomarker assay	Duration (days)	Geometric mean LOEC (µg THC/L)
PAH metabolites					
Cyprinodon variegates	Fish	2	Fixed Fluorescence	35-42	100
Gadus morhua	Fish	3	Fixed Fluorescence	3-30	57.8
Scophthalamus maximus	Fish	1	Fixed Fluorescence	30	16
DNA damage					
Pandalus borealis	Crustacean	2	DNA strand breaks	30-90	21.2
Mytilus edulis	Mollusk	1	DNA strand breaks	210	2.8
Chlamys islandica	Mollusk	1	DNA strand breaks	30	14.4
Strongylocentrotus droebachiensis	Echinoderm	1	DNA strand breaks	210	4
Gadus morhua L.	Fish	3	DNA adducts	24-31	46.9
Cyprinodon variegates	Fish	1	DNA adducts	21	100
Scophthalamus maximus	Fish	1	DNA adducts	30	413
Lysosomal membrane stability					
Chlamys islandica	Mollusc	1	NRRT	30	14.4
Pandalus borealis	Crustacean	2	NRRT	150	9.7
Strongylocentrotus droebachiensis	Echinoderm	1	NRRT	120	29

THC= Total Hydrocarbon Concentration

3.4.2 SSD based on Biomarker response

The SSD is estimated from the biomarker LOECs data and visualized as a cumulative normal distribution function of concentration (logarithmically transformed) and presented in Figure 3-5. Risk is expressed as the *potentially affected fraction* (PAF) which is calculated from:

$$PAF_i = \frac{i}{N+1} \qquad (3.1)$$

where i represents species number and N the total number of species. As a comparison, the SSD from fitness effects are also added into the plot which shows that biomarkers indicate more sensitive responses than the whole organism effects (e.g. growth, reproduction, mortality) (Smit et al., 2009).

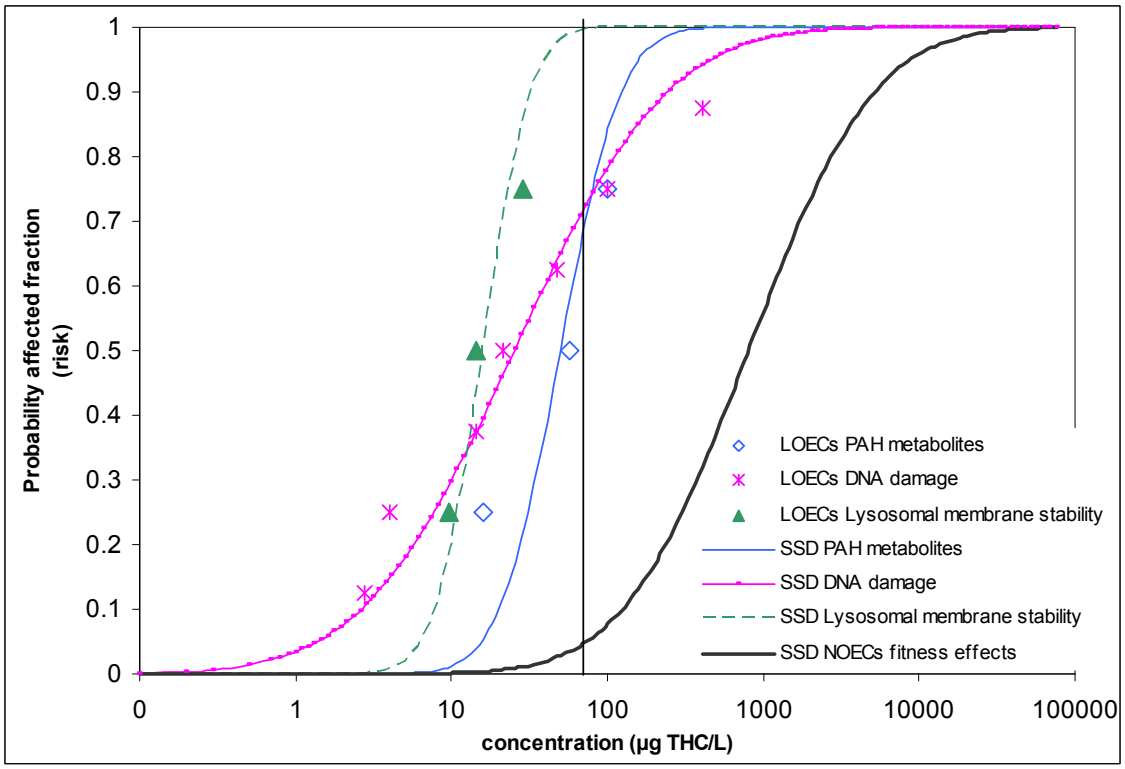


Figure 3-5. Species sensitivity distribution (SSD) curves using normal cumulative distribution based on LOECs from biomarker responses collected from IRIS-Biomiljø and SSD based on NOECs from fitness parameters (Smit et al., 2009).

3.5 Predicting Biomarker Reponses

SSDs based on biomarker response data from the previous step were used to predict the biomarker responses in the Ekofisk region at different distances from the discharge point. For this purpose, several assumptions were made regarding the types of the biomarker that are used.

Assumptions:

1. Lysosomal membrane stability biomarker is considered to be a general measure of stress, therefore the simulation is done based on exposure to all chemicals (naturally occurring and added components) in the discharge.
2. PAH metabolites is considered to be a biomarker of exposure to PAHs and DNA damage is considered to be a biomarker of response to PAHs. Therefore, for these two biomarkers, the simulation is done using chemical groups of polyaromatic hydrocarbons (PAHs) including Napthalenes, PAH 2-3 ring and PAH 4 ring+ as inputs.

Using these assumptions, the risk assessment simulation was performed twice: for all groups of chemicals and for poly-aromatic hydrocarbon (PAH) compunds. The results are then transformed into biomarker response prediction using the SSDs based on biomarker LOECs. Therefore, the predicted biomarker response resulting from the model is in the form of potentially of affected fraction (PAF) of species in the ecosystem.

3.6 Comparison with biomarker responses measured from the field survey

The predicted biomarker responses were then compared with the biomarkers measured from the field survey at Ekofisk. The field biomarker data was obtained from the Water Column Monitoring (WCM) project in 2008 done by IRIS-Biomiljø in collaboration with NIVA, and financed by The Norwegian Oil Industry Association (OLF).

The Water Column Monitoring (WCM) project has investigated the area influenced by produced water discharge in the Ekofisk region since 2006. This project includes the measurement of some core biomarkers in cod and blue mussels located at six observation stations surrounding the discharge at 15 meters depth (see Figure 3-6 for the location of the observation stations). Besides biological responses, the physical environment i.e. current, temperature and salinity were also measured in the field.

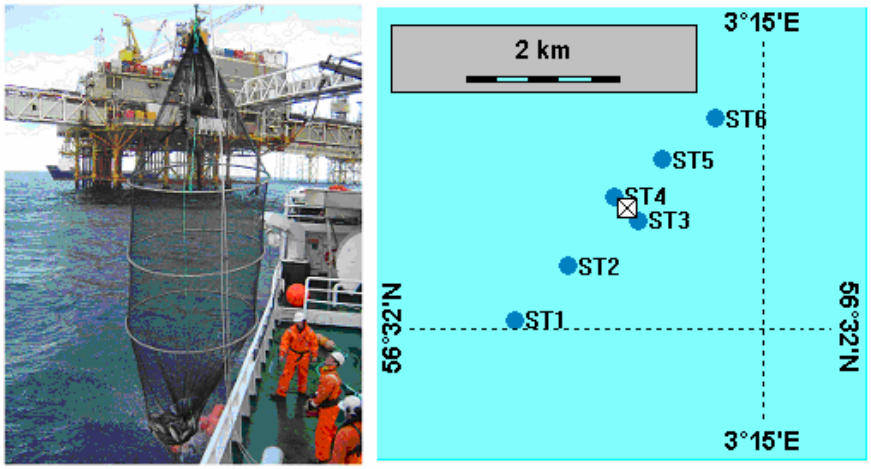


Figure 3-6. Water column monitoring project: deployment of caged cod and blue mussels (left) and Location of 6 stations of cages (right)

For this study, the data collected from WCM includes body burden, lysosomal membrane stability biomarker and micronuclei for mussels, PAH metabolites (both with FF and GC/MS method) and DNA adducts for fish biomarkers. The data from the WCM project in 2008 including the biological responses and physical environment are presented in Appendix C. To have a better validation, wind measurement data in the Ekofisk field from the Norwegian Meteorological Institute (Furevik et al., 2008) is also included.

The biomarker responses for micronuclei and lysosomal membrane stability from 6 observation stations were transformed into contour plots and bubble plots. The contour maps, that are plotted based on the ratio of the responses in each station against the reference value, visualize the level of response. On the other hand, the bubble plots visualize the percentage of individuals for the same species that shows a significant response at different stations compared to the reference value. These contour plots and bubble plots are then overlaid with the predicted biomarker results at the corresponding depth that is at 10-20 meter (second vertical layer).

In addition to the biomarkers, the comparison was also made between the PAH body burden data collected from observation and the predicted body burden calculated using concentration predicted from the model. Using the equation 2.1 (section 2.1.1), the body burden was estimated from the PAH concentration in water based on the bioconcentration factors of PAH compounds in mussel that are available in Table A-1, Appendix A. The PAH concentration results from the model are classified the PAH into three different groups i.e. Naphthalene, PAH 2-3 ring and PAH 4+ ring (list of the compounds included in these groups are available in Table B-2, Appendix B). Therefore, the BCF values from Table A-1 must be simplified in to three corresponding groups. This was done by taking average of different BCF values in the same group. The bioconcentration factors are presented in mg/kg lipid weight, therefore the results have to be translated into ug/kg wet weight, using the average lipid content of 2% of body wet weight in mussel (Baussant et al., 2001)

4 Results

4.1 Environmental risk assessment of produced water discharge in Ekofisk

In the first step of this study, produced water risk assessment in Ekofisk region was simulated in DREAM. The output of this simulation includes the concentration field (Figure 4-1), risk map (Figure 4-2) and also the EIF value and pie chart (Figure 4-3).

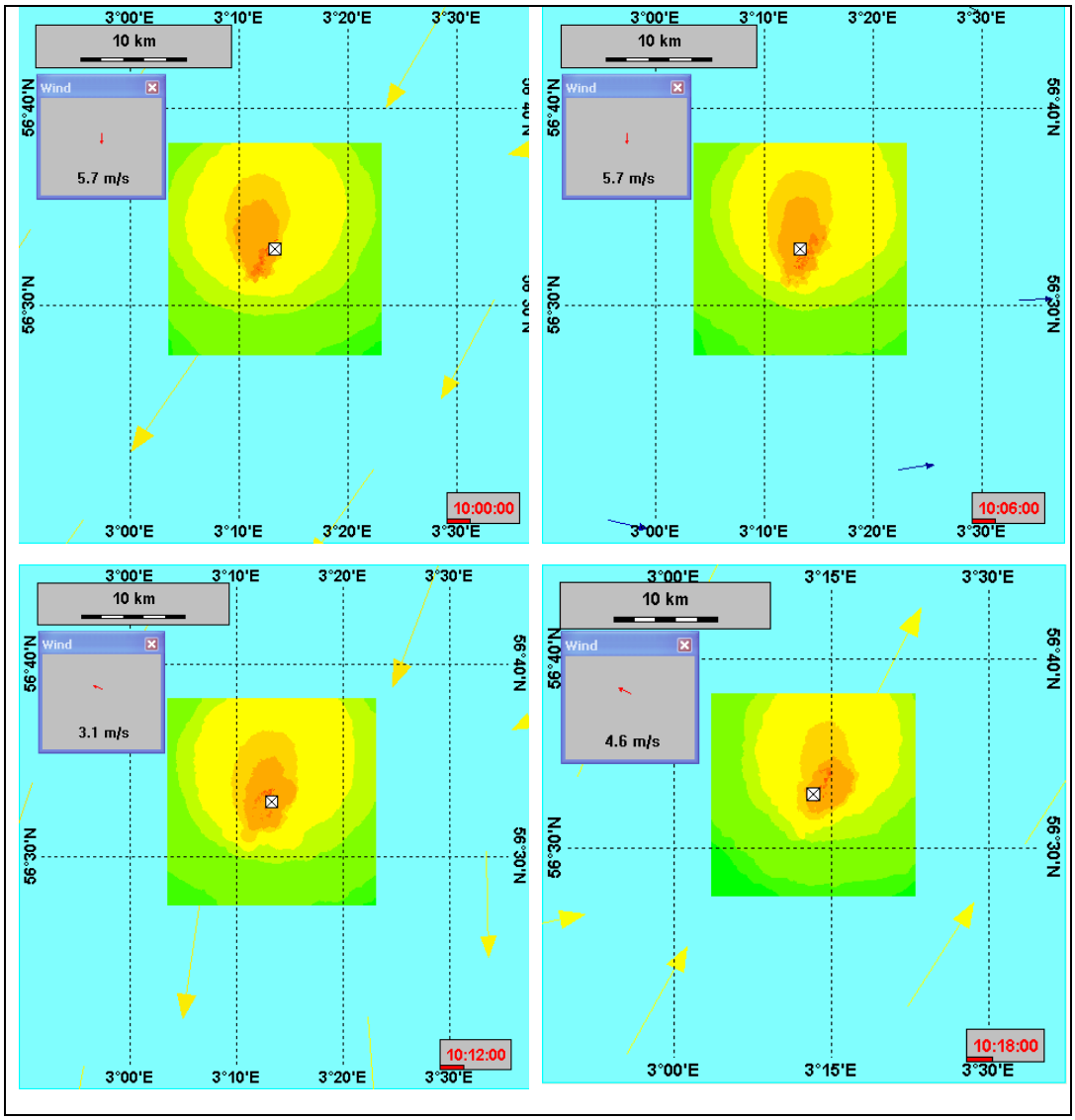


Figure 4-1.Snapshots of concentration field of produced water release during one day (day 10) with 6 hour intervals. The arrows show the current pattern. Wind direction and magnitude are shown in the inserted box.

Figure 4-1 shows the snapshots of the concentration field during one day (day 10) from fate modeling including wind and current pattern. From this picture, it is clear that the contaminant concentration is very dynamic and its distribution follows the movement of current and wind.

Following the concentration field, the risk to the ecosystem due to produced water discharge is also very dynamic. Since decision makers are usually concerned about the maximum risk to the ecosystem that might be caused by the exposure of chemicals ("conservative approach"), then the risk summary map is based on the maximum risk (expressed in percentage) recorded in each cell during the simulation period (Figure 4-2). From the map, it is shown that in the area near the discharge point, there is a significant risk to the ecosystem where the potentially affected fraction of species is greater than 5%.

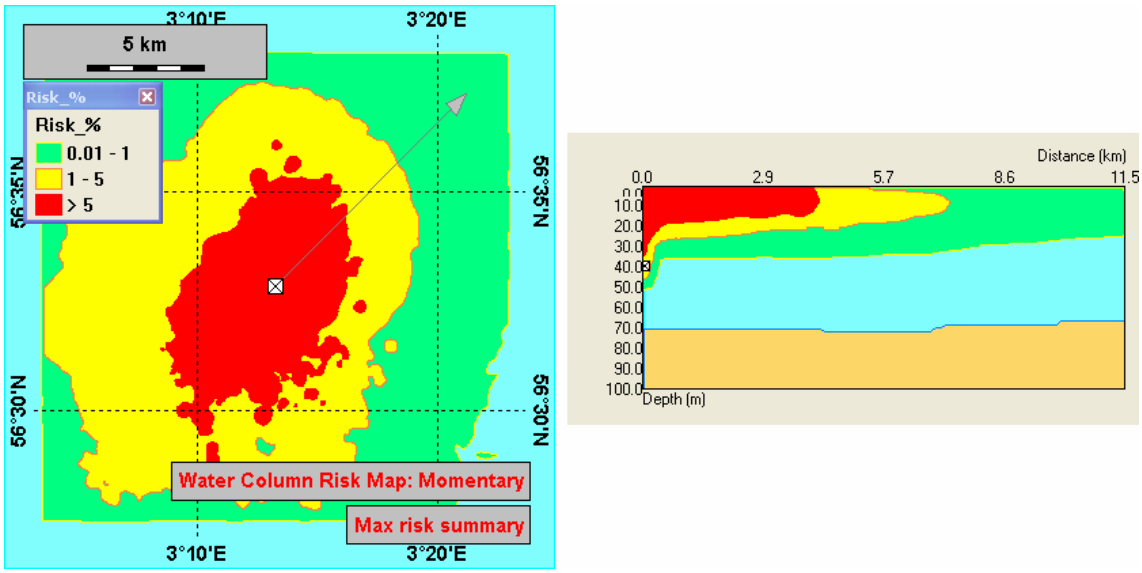


Figure 4-2. Maximum risk in the water column due to produced water discharge in the Ekofisk region. The insert (to the right) shows the vertical distribution along the arrow (in the figure to the left). The red color (risk > 5%) represents PEC:PNEC (RCR) > 1.0.

Besides risk map, the model also calculates the total volume of water that subjected to risk 5% or RCR>1.0 (EIF value). After applying weighting criteria for some groups of chemicals (see Table B-2, Appendix B), the modified EIF value is 718, which means that 71,800,000 m^3 volume of water is subjected to risk greater than 5%. The contribution of the compounds to the total risk is presented in the pie chart (Figure 4-3). From the chart, it is shown that poly-aromatic hydrocarbons (PAHs) and added biocides are the dominant contributors of the total risk to the ecosystem.

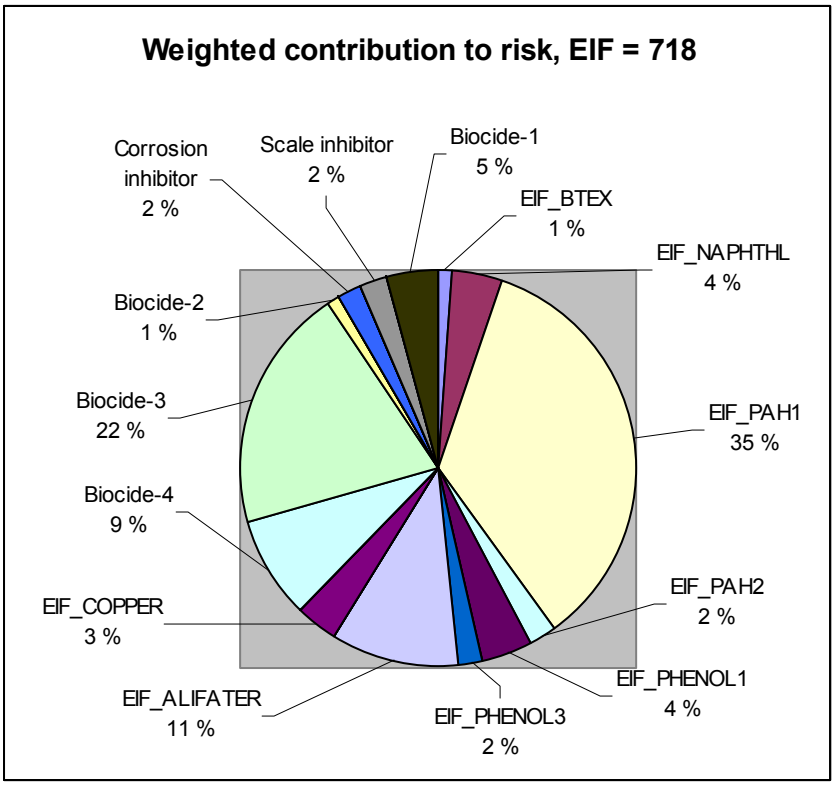


Figure 4-3. EIF value and pie chart of the contribution of chemical groups to the total risk value.

Beside the risk due to all components of produced water discharge, risk assessment due to poly-aromatic hydrocarbons (PAHs) only was also performed (the results are not presented here). Then these results were transformed into the biomarker response (potentially of affected fraction) using the species sensitivity distributions (SSDs).

4.2 Predicted Biomarker Responses

The simulation results for three types of biomarkers: PAH metabolites as a biomarker of exposure, DNA damage as a genotoxicity marker and lysosomal membrane stability as general marker of stress are presented in this section. Figures 4-4 to 4-6 visualize the distribution of the fraction of species that might show responses at the biomolecular level due to produced water exposure in Ekofisk region. The predicted biomarker responses show high levels in the northeast and southwest direction.

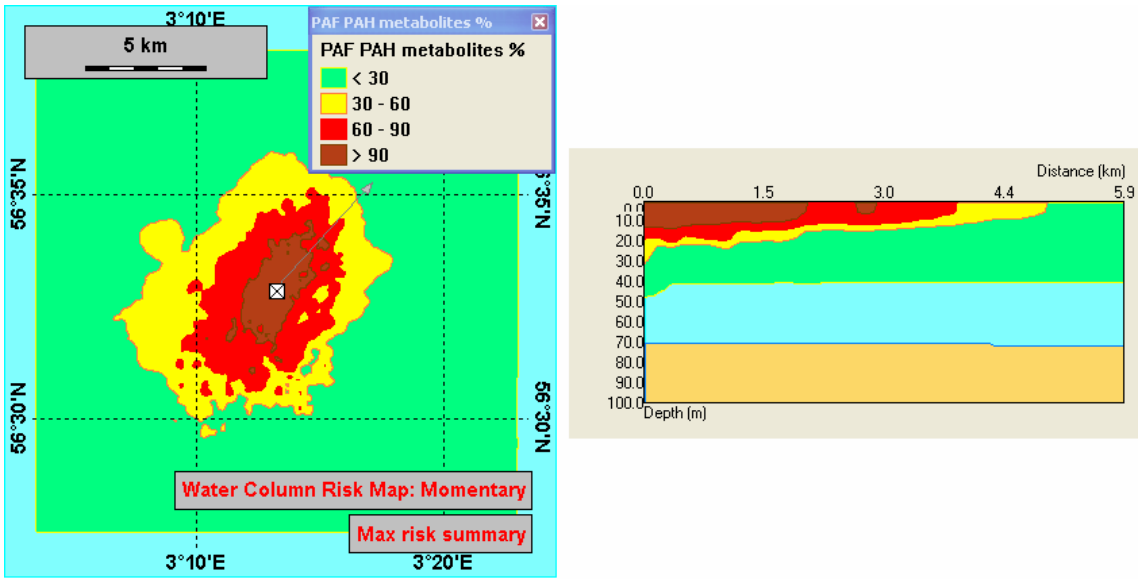


Figure 4-4. Biomarker response prediction at Ekofisk during 30 days period shown as max risk (*potentially affected fraction* [PAF]) in the water column: PAH metabolites.

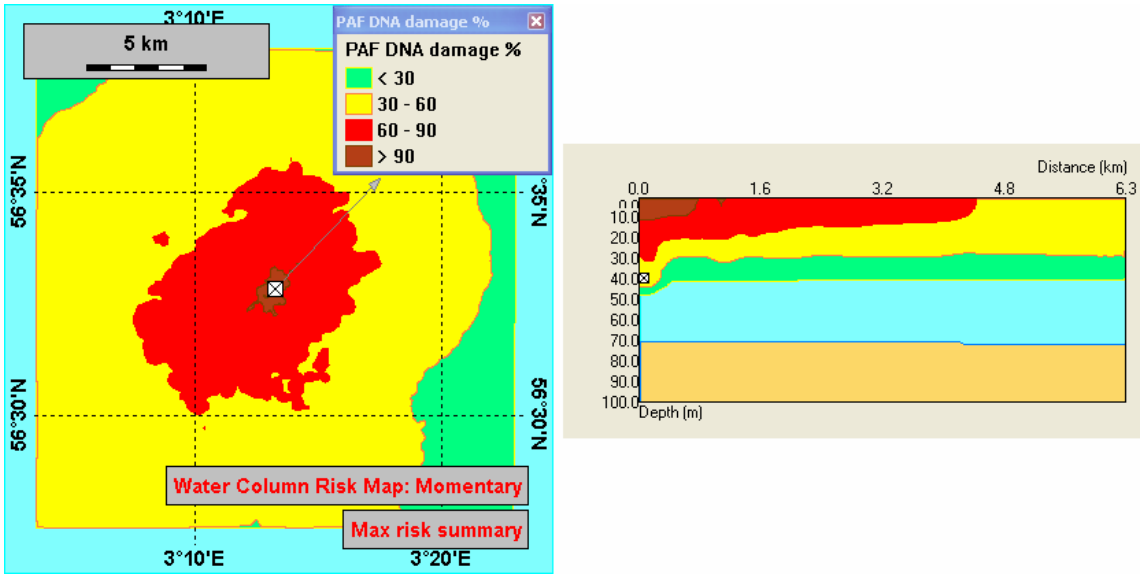


Figure 4-5. Biomarker response prediction at Ekofisk during 30 days period shown as max risk (*potentially affected fraction* [PAF]) in the water column: DNA damage.

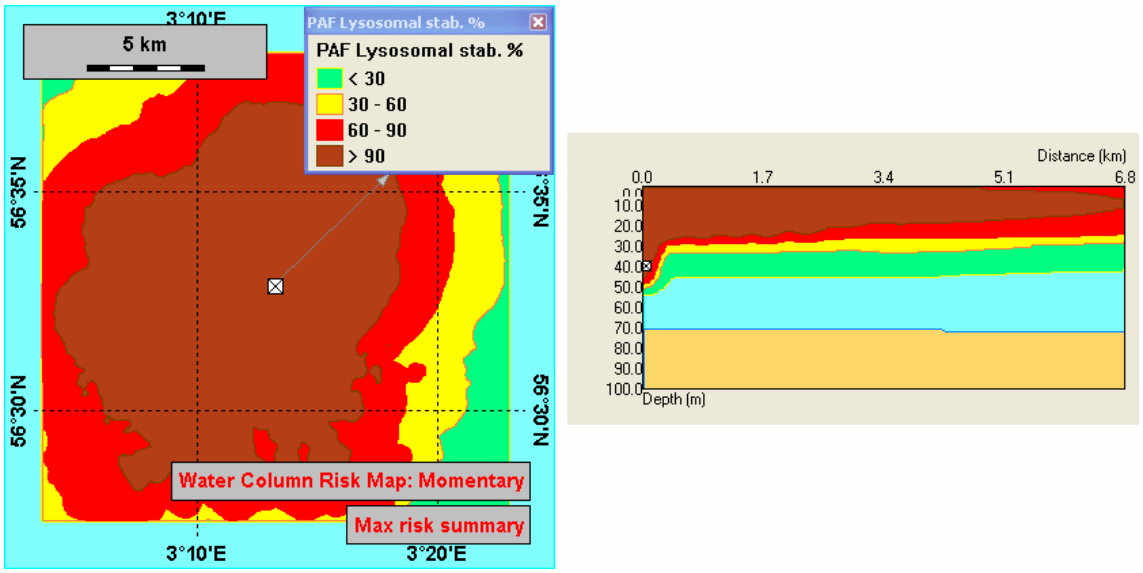


Figure 4-6. Biomarker response prediction at Ekofisk during 30 days period shown as max risk (*potentially affected fraction* [PAF]) in the water column: lysosomal membrane stability.

These results also show that the high biomarker response in lysosomal membrane stability covers wider area. This pattern is determined by the SSD curves (Figure 3-5) used to transform the ecosystem risk into biomarker response that shows lysosomal membrane stability as the most sensitive biomarker.

4.3 Comparison with the Field Measurement

To assess the predicted biomarker responses, the results from the simulations were compared with field measurements. The comparison was made by plotting the field data into contour maps and bubble plots then overlaying them with the predicted results. It has to be remembered this comparison is done in two different units. The model (in risk map) shows the predicted fraction of species that demonstrate alterations at their sub-organismal level. Meanwhile, each line in the contour plot corresponds to the same level of biomarker response (the degree of alterations) compared with reference. For comparison with the bubble plots, it is assumed that both units are comparable, although the bubble plots show the percentage of affected individuals for the same species at six different stations, not a potentially affected fraction of species in an ecosystem. It is done in this way because the availability of field data is very limited.

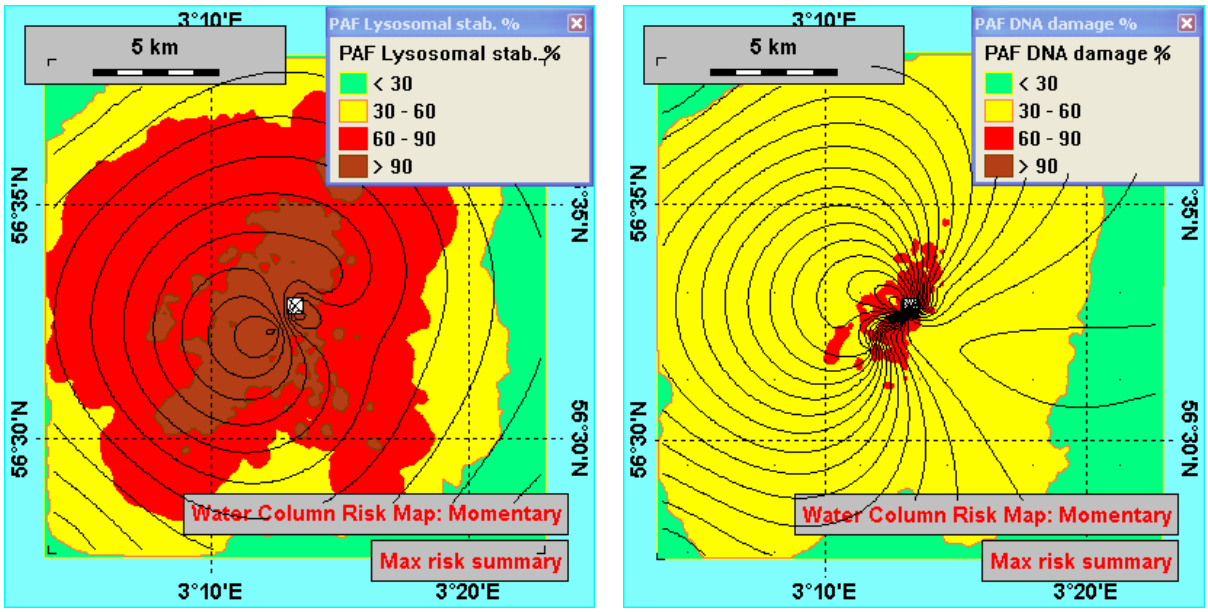
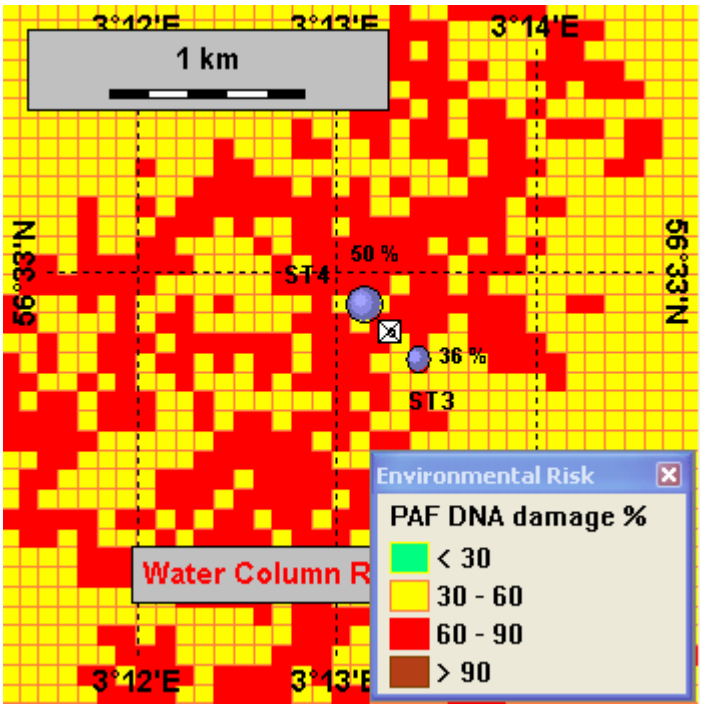


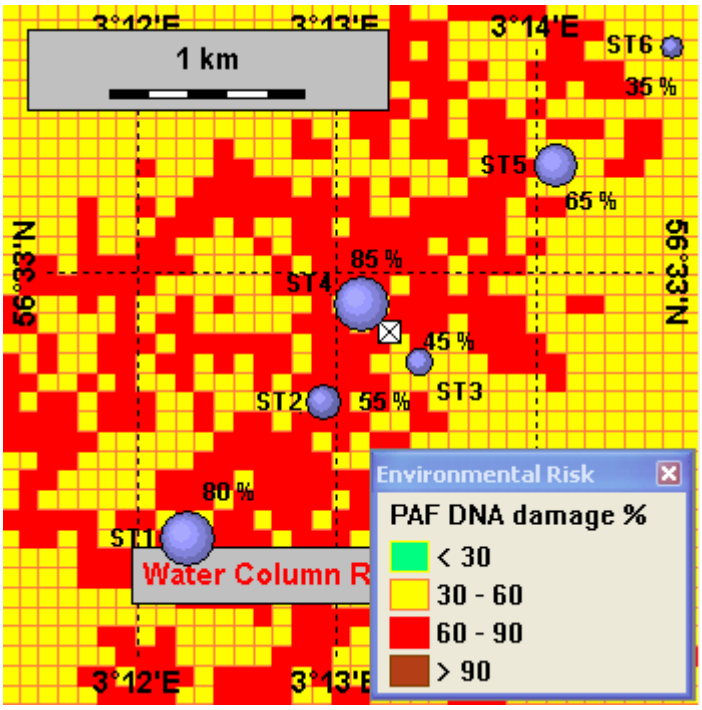
Figure 4-7. Overlay of predicted biomarker response at depth 10-20 m (visualized in risk/response map) with field biomarker measurement (in contour plot) for **(a)** Lysosomal membrane stability and **(b)** Micronuclei. Each line corresponds to the same level of decreasing biomarker responses as increasing distance from discharge point.

The comparison with contour plots is presented in Figure 4-7. Figure 4-8 and 4-10 show the comparison between predicted results and the bubble plots. The values of the validation with the bubble plots are produced in table form (Table 4-1).

Although the response maps and the contour plots are not presented in the similar unit, the comparisons are made to observe the pattern. The contour plots in Figure 4-7 were not given an exact scale, but each line represents the same level of response which is decreasing with increasing distance from the discharge. The biomarker response contour lines show an asymmetrical decreasing pattern which has the lowest response in the southeast of the discharge.



(a)



(b)

Figure 4-8. Comparison of predicted DNA damage biomarker responses from depth 10-20 m (visualized in risk/response map) with measured responses (in bubble plot) of: (a) DNA adduct and (b) micronuclei. The DNA adduct data is only available at 2 stations.

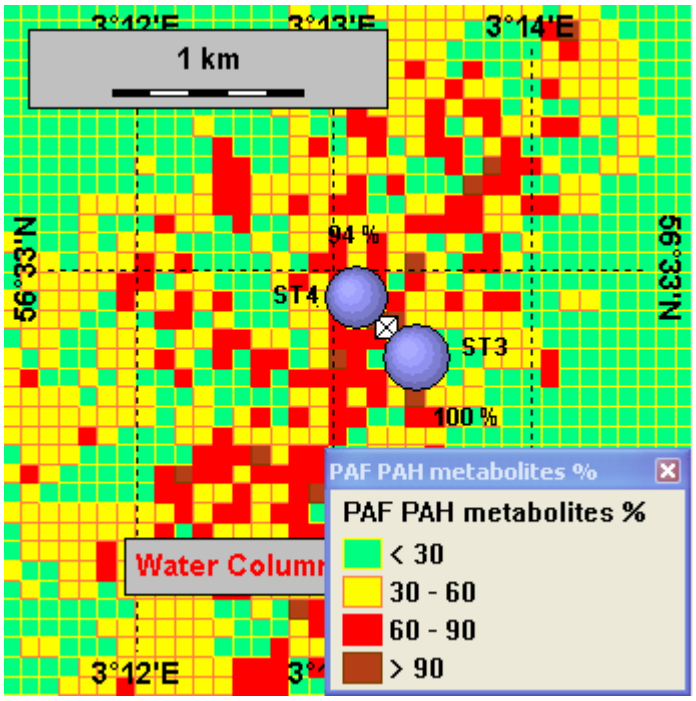


Figure 4-9. Comparison of PAH metabolites biomarker between predicted responses from depth 10-20 m (risk map) with measured responses (bubble plot).

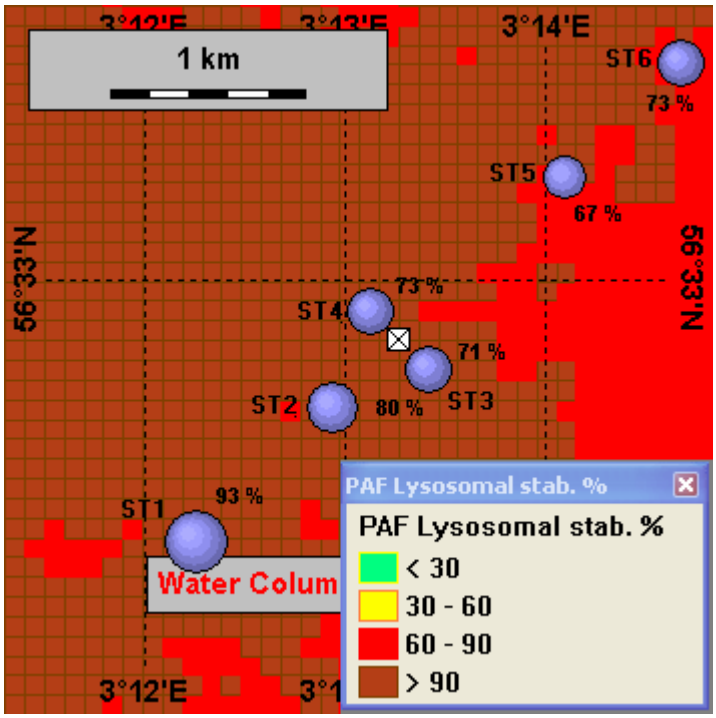


Figure 4-10. Comparison of lysosomal membrane stability biomarker between predicted responses (risk map) with measured responses (bubble plot).

Table 4-1. The comparison of biomarker responses (in probability of affected species) from simulations and observations, assuming that both of them are comparable

(a)Lysosomal membrane stability

Locations	Biomarker responses (%)	
	model	observation
ST1	95	93
ST2	94	80
ST3	93	71
ST4	97	73
ST5	91	67
ST6	85	73

(b)DNA damage (model) –micronuclei (observation)

Locations	Biomarker responses (%)	
	model	observation
ST1	64	80
ST2	63	55
ST3	60	45
ST4	66	85
ST5	57	65
ST6	52	35

(c)DNA damage (model) -DNA adduct (observation)

Locations	Biomarker responses (%)	
	model	observation
ST3	60	36
ST4	66	50

(d)PAH metabolites

Locations	Biomarker responses (%)	
	model	observation
ST3	40	100
ST4	55	94

Table 4-2. Average responses of lysosomal membrane stability and DNA damage biomarkers (both observation and model)

Locations	Average biomarker responses (%)		Deviation = $(Y_{model} - Y_{observation})/Y_{observation}$ (%)
	model	observation	
ST1	80	87	-8
ST2	79	68	16
ST3	77	58	32
ST4	82	79	3
ST5	74	66	12
ST6	69	54	27
		Average=	14

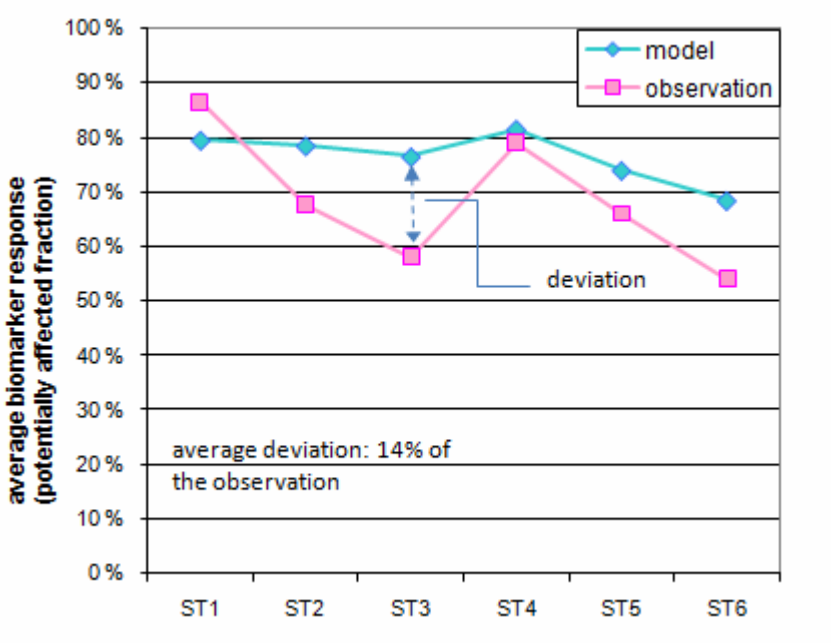


Figure 4-11. Comparison of biomarker responses between model and observation, average results of lysosomal membrane stability and DNA damage

In Figure 4-8 to 4-9, the simulation results are zoomed and then compared with the percentage of affected individuals in the same species (presented as bubble plots). In a brief look, the predicted response maps show some agreements with the bubble plots pattern. The values of the predicted responses and the measured responses are presented in tables (Table 4-1 (a)-(d)). The predicted results (PAF of species) are calculated by taking the average of the corresponding cell of each station and its surrounding cells.

The average responses of lysosomal membrane stability and DNA damage-micronuclei biomarkers in each corresponding station are calculated for both predicted and observed, and the results are presented in the Table 4-2 and Figure 4-11. In Table 4-2 the deviations between the predicted and observed results were also calculated. The deviations were defined as the percentage of the difference between the model and the observation (not to be confused with the biomarker responses that are also expressed in percentage). The results show that the model and the observation have the same trend. It is also found that the model gives, in average, 14% higher response than the observation, except at ST1 which is about 8% lower than the observation.

Beside the biomarker response comparison, the body burden data of PAHs in mussels from field measurement were also collected to study the internal exposure. The data were then compared with the concentration data from the model. This is shown in Figure 4-12. The figure shows a good comparison between the model and observation, especially at the stations far from the discharge point (ST1, ST5 and ST6). Meanwhile at the locations near the discharge point (ST2, ST3 and ST4), the comparison was rather poor.

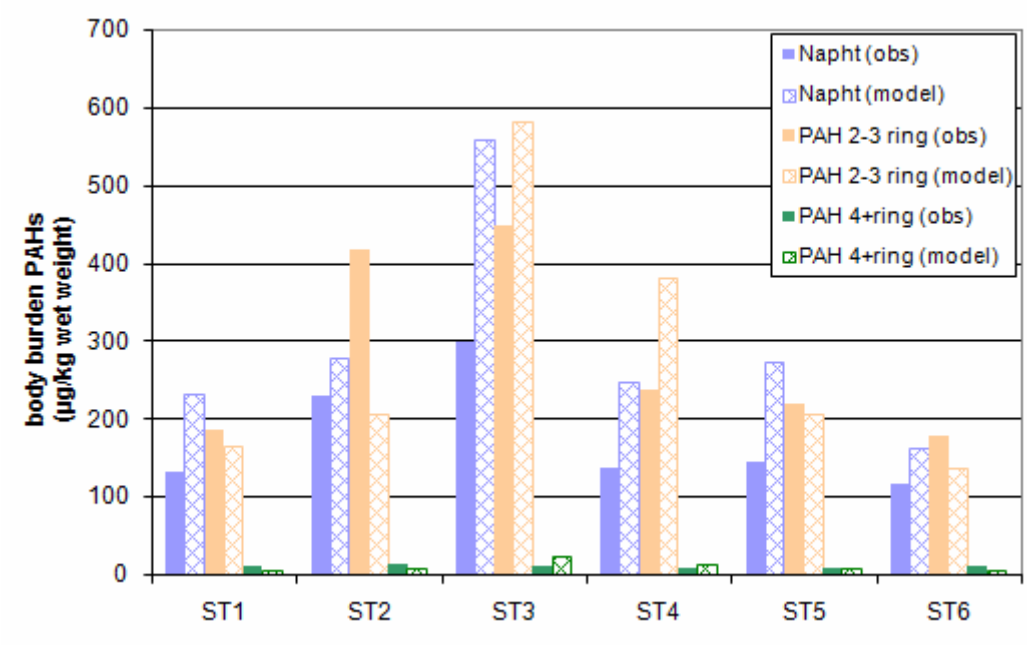


Figure 4-12. Comparison of predicted body burden calculated using maximum concentration from model and body burden data from observation. PAH body burden in mussels are measured in µg/kg wet tissue.

5 Discussion

In establishing the link between the risk prediction model (DREAM) and biological monitoring, the DREAM model is utilized to predict the three types of biomarker responses (i.e. lysosomal membrane stability, PAH metabolites and DNA damage) then the results are compared with the biomarker responses from field measurement.

The methodology of predicting biomarker response in DREAM in this study utilizes the species sensitivity distribution curves based on biomarker's LOECs or so-called biomarker response distributions (BRDs). Therefore, the accuracy of the SSD curves is essential to produce good results. The accuracy of the curves mainly relies to the number of data and the accuracy of the data. The biomarker's LOECs obtained from laboratory experiments are currently only available for a few species. Another source of uncertainty in the SSD curves is that few LOEC data collected from the experiments were the lowest concentration tested. This means that the true LOECs could be considerably lower than the value obtained.

Despite the remaining uncertainties, SSD curves for different biomarker responses (Figure 3-5) show a clear pattern: the biomarker responses are more sensitive than the whole organism effect (fitness). Lysosomal membrane stability is the most sensitive marker compared to others. It might be because LOECs of lysosomal membrane stability are mostly taken from exposures to invertebrates (although it can also be measured on fish) and fish, in general, have more efficient detoxification defenses than invertebrates. On the other hand, the PAH metabolites biomarker which is a biomarker exposure is shown to be less sensitive than the biomarker effect, DNA damage. This is because the PAH metabolites in bile is only available for fish (vertebrates). Meanwhile, the DNA damage SSD is based on the exposures of both invertebrates and vertebrates.

The prediction of biomarker responses using the DREAM model is determined by how the model works. The DREAM model enables a dynamic risk prediction and also takes into account the different properties in a complex mixture. In the model, the predicted risk is expressed as the potentially affected fraction of species in the ecosystem, where the risk values above 5% have been considered to be unacceptable. Therefore, the results of predicted biomarker response are expressed as the fraction of species in the

ecosystem that show alterations in a certain biological marker. However, for validation or comparison purpose, the field measurement biomarkers cannot be translated into the same unit as the model, since the available field data only consist of two species which are not sufficient to represent an ecosystem. The comparison was done in two possible ways: comparing the model with pattern of the responses measured from field (expressed in contour plots) and comparing the model with percentage of affected individuals for the same species at six different points (presented as bubble plots).

The simulation results show a common pattern that is the high values of response (probability affected fraction) are mainly distributed in the northeast and southwest direction. The observation results for lysosomal membrane stability, micronuclei and DNA adduct biomarkers show that ST4 is higher than ST3 and the south west direction shows higher response (potentially affected fraction of species) than the north east direction. This pattern can be explained by the current and wind distribution in the region as the contaminant fates are highly determined by the physical environment. The dominant current pattern in Ekofisk is distributed in northeast and southwest directions (see Figure C-1(a), Appendix C). In addition to the current effect, the southwest side of the discharge point is subjected to higher wind velocity (see Figure C-2, Appendix C) which might explain higher response in that direction compared to northeast direction.

The average responses of lysosomal membrane stability and DNA damage-micronuclei biomarkers in each corresponding station are calculated for both predicted and observed, and the results are presented in the Table 4-2 and Figure 4-11. In Table 4-2 the deviations between the predicted and observed results were also calculated. The average biomarker response comparison (Figure 4-11) shows that in general the model and the observation have a similar trend. The result also shows that the model gives about 14% higher response than the observation, except at ST1 which is 8% lower than the observation.

The higher response of the predicted biomarkers may be due to the fact that the response map from the model is based on the highest risk recorded for the simulation period. Since the simulation results are based on the highest response during the 30 days period, those prediction values must be treated carefully. It has to be remembered that the actual exposure processes are very dynamic. One maximum response value might not be able to adequately represent the potential biological response at one location.

To obtain more representative predicted values in relation to the field measurement, the comparison could instead have been based on the average values throughout the simulation period or on the last part of it. This is not done, as it would require modification of the standard calculation of the model output, which would be too time demanding for this thesis project.

For the results on PAH metabolites observation, the average level of response (see Figure C-3, Appendix C) and also the percentage of affected species (bubble plot in Figure 4-9 or values in Table 4-1 (d)) show quite a different pattern than the other biomarkers. The percentage affected individuals of PAH metabolites from field measurement in ST3 is slightly higher than ST4 which is contradictive to the biomarker prediction result. It may be due to the fact that the results from the biomarker response prediction in Table 4-1(d) have been smoothed with the surrounding cells.

PAH metabolites biomarker is mostly used as biomarker of exposure since it can provide information of the recent (ongoing) exposure of fish to poly-aromatic hydrocarbons (PAHs). As mentioned earlier, the exposure of produced water in one location is very dynamic due to the physical environment (i.e. current and wind circulation). Therefore, the possible explanation for this condition is that around the time when the samples were taken, the ST3 were subjected to higher concentration than in ST4.

In general, the time variable or duration of the exposure is important in interpreting the biomarker responses. For enzymatic biomarkers in fish, the process induction and recovery of the responsive system might last for some days up to few weeks, whereas some other biomarkers may require longer time (Beyer and Bamber, 2004). This is will also be the case for PAH metabolites which will vary dynamically with the enzymatic processes of metabolism, while DNA damage will be more accumulated and less dynamic in its formation and recovery process as its link to metabolism process is slightly less direct.

As for the model, although the DREAM model is able to estimate the time variable exposure process in the biomarker response prediction, it cannot accommodate the duration variable in the response formation and recovery processes.

As the biomarker responses are highly determined by the level of exposure, in addition to the biomarker response data, the body burden data of PAHs in mussels were also collected. The result is then compared to the maximum concentration profile in 6 stations which have been transformed into the same unit as the measured values by using the bioconcentration factors from experiments (Baussant et al., 2001) (Figure 4-12). The figure shows a good comparison between the predicted and observed body burden at the stations far from the discharge point (ST1, ST5 and ST6), meanwhile at the locations near the discharge point (ST2, ST3 and ST4), the comparison was rather poor. The predicted PAH body burden results are also based on the maximum concentration recorded during the simulation, therefore, the results depend greatly to the concentration profile. At the location near the discharge source, the concentration varies greatly in time and ST3 and ST4 could be subjected to a very high concentration in a very short time. Meanwhile, at the locations further from the discharge source the concentration profiles do not have so much fluctuation compared to the area near the discharge point, thus give more stable results and a better comparison with the observation. This condition suggests that the internal exposure of contaminants in biota is influenced by the dynamic concentration of the contaminants.

It has to be noted, in performing the biomarker response prediction, several assumptions and simplifications have been applied including the time frame of the simulation. The simulation is performed for a 30 days period based on wind and current data in May 1990, which are the standard wind and current inputs, while the field data are based on 6 weeks observation from April - May 2008.

The salinity and temperature inputs for the simulation were based on the actual field data. This was done to study more about the vertical profile of the discharge pattern in relation to the near-field module. The results show that the vertical density profile (represent by salinity and temperature profile) which may cause a vertical stratification of water mass does not give any considerable influence to the concentration profile, since the concentration is more accumulated at the surface. This is due to the fact that the produced water discharge usually has a quite high temperature and also includes hydrocarbons and causing the produced water to have very low density and thus spreading up to the surface.

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6 Conclusion

The aim of this study is to investigate the possibility of linking a risk prediction model with biomarker responses which was done by utilizing the DREAM model to predict biomarker responses applying the species sensitivity distribution (SSD) approach.

From the results, predicting biomarker responses using the DREAM model can be done with some assumptions applied. It is also shown that the model could predict similar trend with biomarker responses measured at different distance from the discharge point. In this study, the predicted biomarker responses give about 14% higher value compared to observation. It may be related to the fact that the model applies the conservative approach (based on the maximum risk value) and therefore the model cannot accommodate the duration variable in the biomarker response formation and recovery processes which may differ in biomarkers which is also the case for the body burden.

Despite the remaining uncertainties and limitations, this study could provide some general backgrounds for the study of linking the risk prediction with the monitoring. From this study it can also be concluded that the results in biomarker responses prediction is determined by three factors: the reliability of the SSD approach, the model limitations (how the model works) and also the available field data for validation. Therefore, in future, there are several improvements that could be applied for studies related to this topic:

- The accuracy of the curves mainly relies on the number of data and their accuracy. In this study, the SSD curves are mainly constructed based on LOECs from few species. Including LOEC data from more species that represent more diverse taxonomy is one way to increase the reliability of the SSD curves. Some LOEC data from the experiments used in this study are actually the lowest concentration tested, which makes them to be rather imprecise data. Therefore, improvement in the accuracy/quality of the LOEC data is also important in improving the accuracy of the SSD curves.

- So far, the simulation results selected from the model are based on the maximum risk, since the risk summary results are based on maximum exposure during the simulation. It would be interesting to compare the observed results with the average summary results from the model.
- It is also suggested to perform the simulation in the same time frame with the observation to increase the consistency between the prediction and observation.
- In this study, the results (predicted affected fraction of species) from the model are compared with the percentage of affected individuals in the same species from observation, due to the limited observation data available. Therefore, increasing the species number from observation would make it possible to make the validation/comparison in exactly the same unit.

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Appendix A

Table A-1. Lipid base bioconcentration factors (BCF) in sample tissues and devices (SPMD=semi-permeable membrane device) of blue mussel (*Mytilus edulis*) and cod (*Scopthalamus maximus*) (Bausssant, 2001).

			BCF lipid weight ($\times 10^4$)	
Compound	log K_{ow}[a]	SPMD	*Mytilus edulis*	*Scophthalmus maximus*
Naphthalene	3.34	1.86	1.33	1.78
C1-naphthalenes	3.88	4.49	2.76	4.17
Acenaphthene	3.95	5.56	6.54	0.55
Acenaphtylene	4.1	15.84	14.46	5.33
C2-naphthalenes	4.37	8.95	8.30	5.22
Fluorene	4.21	6.12	5.09	3.31
C3-naphthalenes	4.86	12.84	15.98	2.18
Phenanthrene	4.57	16.55	14.66	1.03
Anthracene	4.58	—	—	1.72
Dibenzothiophene	4.38	32.19	21.15	3.21
C1-phenanthrenes	5.1	19.26	23.39	0.16
C1-dibenzothiophenes	—	37.29	33.43	0.26
Fluoranthene	5.1	—	—	0
Pyrene	5.1	6.53	33.43	0
C2-phenanthrenes	—	18.79	26.48	0.02
C2-dibenzothiophenes	—	35.28	37.66	0.03
Benzo[*a*]anthracene	5.67	—	—	0
Chrysene	5.71	4.36	20.76	0
C1-chrysenes	—	3.24	10.69	—
Benzo[*b*]fluoranthene	6.4	—	—	—
Benzo[*k*]fluoranthene	6.5	—	—	—
Benzo[*a*]pyrene	6.3	—	—	—
C2-chrysenes	—	5.35	9.75	—
Indeno[*1,2,3,c,d*]pyrene	6.92	—	—	—
Benzo[*g,h,i*]perylene	7	—	—	—
Dibenzo[*a,h*]anthracene	6.71	—	—	—
Benzo[*b+k*]fluoranthene	—	—	—	—

Appendix B

Table B-1. Produced water compound groups representing naturally occurring components and man-added components with their PNEC values and weighing factors (OLF, 2003)

Group	Main group	Representative compound	PNEC values (ppb)	Weighing factor
1	BTEX	Benzene	17	1
2	Napthalenes	Napthalene	2,1	1
3	PAH 2-3 ring	Phenanthrene	0,15	1
4	PAH 4 ring+	Benzo[a]pyrene	0,05	2
5	Phenols C0-C3	Phenol	10	1
6	Phenols C4-C5	Pentylphenol	0,36	1
7	Phenols C6-C9	Nonyphenol	0,04	2
8	Aliphatic hydrocarbons	Hepthane	40.4	2
9	Metals 1 Zinc Copper Nickel		 0,46 0,02 1,22	 1 1 1
10	Metals 2 Lead Cadmium Mercury		 0,182 0,028 0,008	 1 1 1
11	Corrosion inhibitor		HOCNF specific data	see Table A-4
12	Biocide		HOCNF specific data	see Table A-4
13	Scale inhibitor		HOCNF specific data	see Table A-4
14	Anti foam		HOCNF specific data	see Table A-4
15	Emulsion breaker		HOCNF specific data	see Table A-4
16	Flocculant		HOCNF specific data	see Table A-4
17	H_2S scavanger		HOCNF specific data	see Table A-4

Table B-2. List of the compounds included in groups of naturally occurring components of produced water (OLF, 2003)

Main groups	Compounds
Naphthalene	• Naphthalene • C1- Naphthalene • C2-Naphthalene • C3- Naphthalene
PAH 2-3 ring	Compounds on the EPA 16 PAH list with 2-3 rings, other than Naphthalenes: • Acenaphthylene • Acenaphthene • Fluorene • Phenanthrene, including C1-C3 alkylhomologues • Anthracene • Dibenzothiphenes, including C1-C3 alkylhomologues
PAH 4+ ring	Compounds on the EPA 16 PAH list with 4 rings or more • Fluoranthene • Pyrene • Chrysene • Benzo(a)anthracene • Benzo(b)fluoranthene • Benzo(k)fluoranthene • Benzo(a)pyrene

	• Indeno(123,cd)pyrene • Dibenzo(ah)anthracene • Benzo(ghi)perylene
Phenol C0-C3	Phenols C1-C3 alkylhomologues: • Phenol • C1-Phenols o o-cresol o m-cresol o p-cresol • C2-Phenols o 2,5-Xylenol o 3,5-Xylenol o 2,4-Xylenol o 4-Ethylphenol o other C2-phenol alkylhomologues defined by analytical method • C3-Phenols o 2-n-Prophylphenol o 2.3.5-Trimethylphenol o 4-n- Prophylphenol o 2.4.6-Trimethylphenol o other C3-phenol alkylhomologues defined by analytical method
Phenol C4-C5	C4-C5 alkylphenol homologues: • C4-Phenols o 4-tert-Butylphenol o 4-iso-Propyl-3-Methylphenol o 4-n- Butylphenol o other C4-phenol alkylhomologues defined by analytical method • C5-Phenols o 2-tert-Butyl-4-Methylphenol o 4-tert-Butyl-4-Methylphenol o 4-n-Pentylphenol o other C4-phenol alkylhomologues defined by analytical method
Phenol C6+	• Sum C6-Phenols o 2,6,-Di-iso-Propylphenol o 2,5,-Di-iso-Propylphenol o 4-n-Pentylphenol o 2-tert-Butyl-4-Ethylphenol o 6-tert-Butyl-2,4-Dimethylphenol • Sum C7-Phenols o 4-n-Hepthylphenol • Sum C8-Phenols o 2,4,-Di-sec- Butylphenol o 4-tert-Octylphenol o 4-n-Pentylphenol o 2,6-Di-tert- Butylphenol o 2,6-Di-tert-Butyl-4-Methylphenol o 4-n-Octylphenol • Sum C9-Phenols o 2-Methyl-4-tertt-Octylphenol o 4-n-Nonylphenol o 4,6-Di-tert-Butyl-2-Methylphenol o 2,6-Dimethyl-4-(1,1-Dimethylpropyl)phenol o 4-(1-ethyl-1-methylpropyl)-2-methylphenol

Table B-3. Standard biodegradation rates for produced water compounds (Johnsen et al., 2000)

Group	Main group	Biodegradation rate ½ life (days)
1	BTEX	0,5
2	Napthalenes	1,5
3	PAH 2-3 ring	17
4	PAH 4 ring+	350
5	Phenols C0-C3	1,2
6	Phenols C4-C5	10
7	Phenols C6-C9	
8	Aliphatic hydrocarbons	60
9	Metals 1	No degradation
10	Metals 2	No degradation
11-n	Poduction chemicals	HOCNF (BOD 28d) specific data

Table B-4. Weighing criteria in EIF based on bioaccumulation and biodegradation potential (Johnsen et al., 2000)

Biodegradation (BOD, 28 days test)	Bioaccumulation (log P_{ow})		
	<3	3-5	>5
>60%	1	1	1
20-60%	1	2	2
<20%	2	2	4

Appendix C

C.1. Physical Environment Data

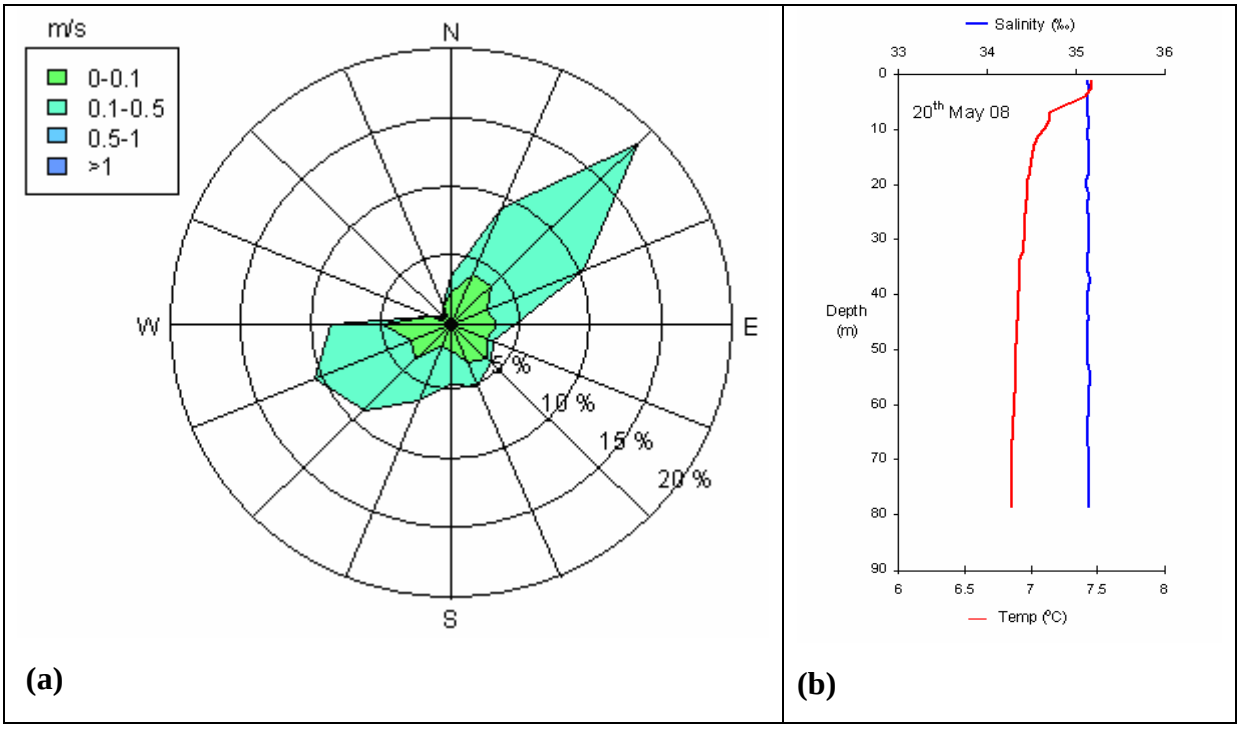


Figure C- 1. Physical environment data from WCM 2008: **(a)** Ocean current distribution; **(b)** Salinity and Temperature Profile

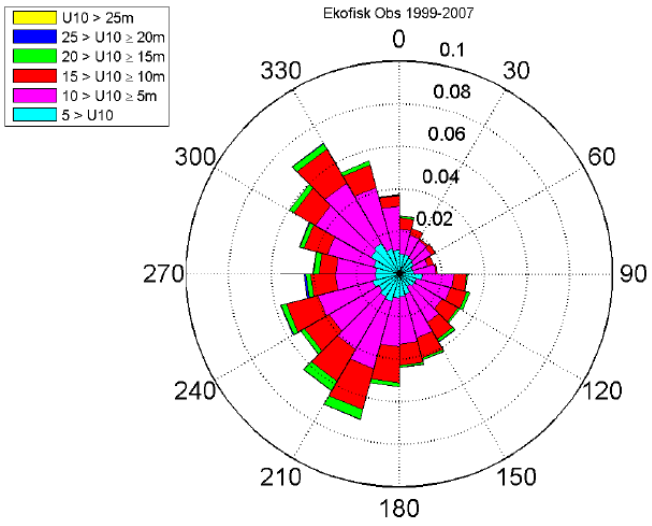
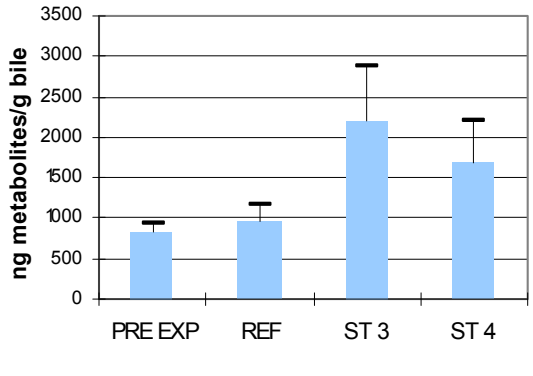
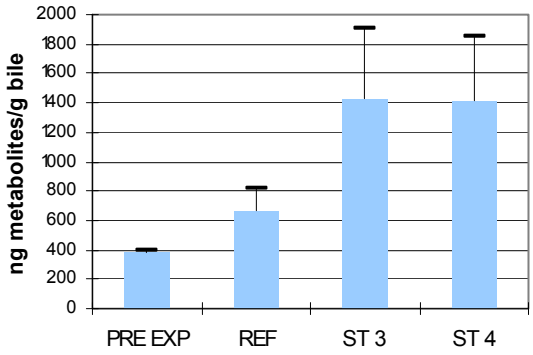


Figure C-2. Wind rose for in-situ observation during 8.5 year period at Ekofisk from Norwegian Meteorological Institute: www.met.no (Furevik et al., 2008)

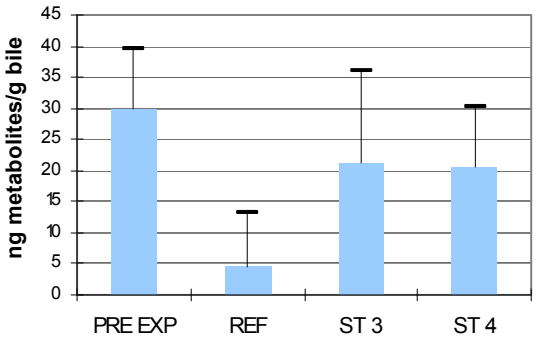
C.2. Biomarker responses from Water Column Monitoring 2008



(a)



(b)



(c)

Figure C-3. PAH metabolites biomarker data from cod are only available from 2 stations (ST3 and ST4). PAH metabolites is measured in ng metabolites/ g bile. The data presentation is divided into three groups of polyaromatic hydrocarbons (PAHs) in accordance with the EIF concept; (a)Napthalenes, (b)PAH 2-3ring, (c)PAH 4ring+ (Sundt et al., 2008)

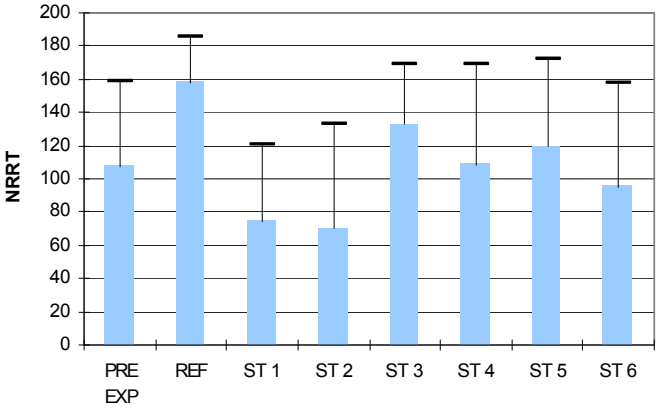


Figure C-4. Lysosomal membrane stability biomarker from mussels in 6 stations, REF is reference value and PRE EXP is condition before experiment. Lysosomal membrane stability is measured in Neutral Red Retention Time (NRRT) assay (Sundt et al., 2008).

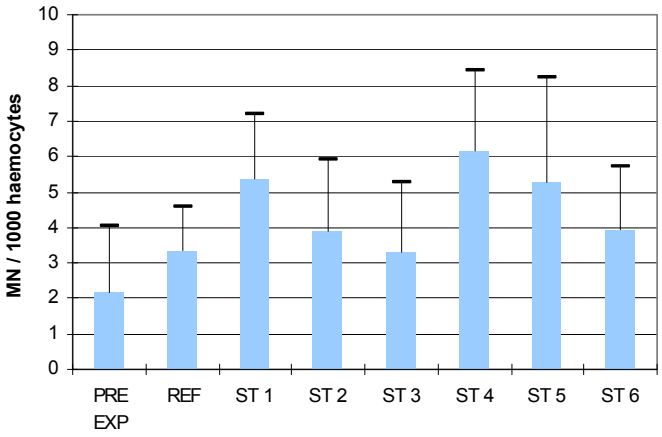


Figure C- 5. Micronuclei biomarker from mussels in 6 stations, REF is reference value and PRE EXP is condition before experiment (Sundt et al., 2008).

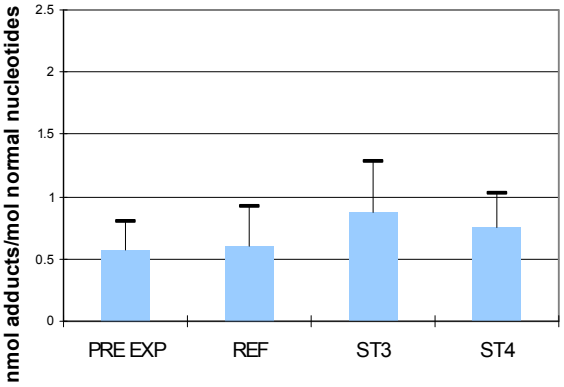


Figure C-6. DNA adduct responses from fish in 2 stations, REF is reference value and PRE EXP is condition before experiment. DNA adduct response is measured in nmol adduct/mol normal nucleous (Sundt et al., 2008)

C.3. Body Burden Data from Water Column Monitoring 2008

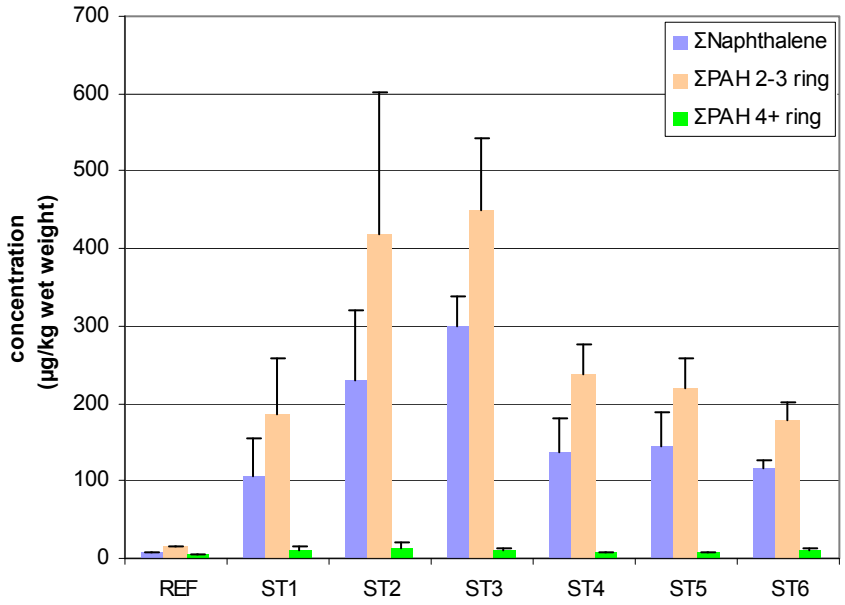


Figure C-7. Body burden data for PAHs in mussels (Sundt et al., 2008)