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# Field Based Predicted No Effect Concentrations (F-PNECs) for macro benthos on the Norwegian Continental Shelf

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Report

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# **Background and summary**

This report is a result of a project initiated within the ERMS project and has been followed up by Statoil's Research Center in Trondheim. The scope has been to produce Field Based Species Sensitivity Distributions (F-SSDs<sup>1</sup>) and Field Based Predicted No Effect Concentrations (F-PNECs) for macro benthos living on the Norwegian Continental Shelf. The data is from the Norwegian Oil Industry Association (OLF) database, the MOD-database; containing abundance data for more than 2000 species and concentration data for more than 20 substances. The F-PNECs will be used to validate PNECs available from literature in order to assess whether these data are protective to the assemblage in the natural ecosystem.

The project has been divided into two parts: part I covers the work carried out by University of Oslo (UoO) in the period of four months starting late March 2004. Part II was an extension of part I for UoO and previous projects performed by Akvaplan niva and MUST as (ERMS Task 5). For the University of Oslo, part II covers work carried out in the period of 1 month, starting mid November 2004. The UoO have also cooperation with The University of Hong Kong and City University of Hong Kong. One paper is in press (see references), and other papers are under preparation.

The main F-PNECs are summarized in Table A. The F-PNECs is derived from F-SSDs of sensitive macro benthos using bootstrap technique (see methods). The F-SSD is a measurement of the benthic community response to a specific substance in the presence of other substances in the sediment, taking into account physical such as depth and sediment characteristic and biotic factors such as predation and competition. The F-PNECs for dataset 1 are derived from the 191 most common species in the MOD-database. The F-PNECs for dataset 2a, 2b and 2c is derived from all species in the MOD-database (given that they fulfil the criteria's about occurrence and abundance; see Chapter 4 page 12) for three different grain size intervals; (1) "mud", (2) "mud- fine sand" and (3)"fine sand – sand", respectively. The F-PNECs in Table A is adjusted for non-sensitive species, i.e. the x expresses the protection level that in theory should protect 95 % of the sediment dwelling macro benthos community at the Norwegian Continental Shelf (Formulae 3, page 10).

<sup>&</sup>lt;sup>1</sup> The terms F-SSDs and F-PNECs is used in this report to differentiate them from SSDs and PNECs estimated from laboratory experiments, which follow strict calculations rules to derive its value (see Box 1.1, 1.2 and 1.3).

**Table A:** Medians for adjusted F-PNECs, i.e. the actual concentration level below which harmful effects on the benthos community are unlikely to be observed, derived form F-SSDs using bootstrap method. Concentration in mg kg<sup>-1</sup>, dry weight (ppm). Dataset 1 is the 191 most common species. Dataset 2a, 2b, and 2c is for the grain size intervals: "mud", "mud – fine sand" and "fine sand –sand", respectively. See formulae 3 on page 19 for an explanation of x.

Substances	Dataset 1	x	Dataset 2a	X	Dataset 2b	X	Dataset 2c	X	Mean Dataset 2
Substances	F-PNECx	(%)	F-PNECx	(%)	F-PNECx	(%)	F-PNECx	(%)	F-PNECx
Ba	1718	13	2645	13	2263	10	1951	9	2286
Cd	0.039	9	0.052	9	0.046	8	0.089	6	0.062
Cr	9.801	8	14.14	7	7.901	8	8.211	9	10.08
Cu	6.438	8	7.374	9	5.725	9	6.291	9	6.463
Hg	0.023	10	0.034	9	0.174	7	NA	NA	0.104
Pb	11.50	9	18.65	9	12.76	9	12.55	8	14.65
Zn	26.59	8	34.00	9	30.42	10	28.49	8	30.97
ТНС	100.2	7	71.2	9	130.5	8	99.1	8	100.3
NPD (sum)	0.205	9	0.244	9	0.217	10	0.089	6	0.183
PAH (sum)	0.075	8	0.187	10	NA	NA	0.129	9	0.158
PAHc (sum)	NI	7	0.238	10	0.191	10	0.094	9	0.174
Decalins	8.810	13	10.45	8	20.22	11	16.34	8	15.67

NI means that the substance has not been investigated and NA means that there were not adequate data to derive F-SSDs (see criteria's in Chapter 4 on page 12).

# **1** Introduction

This report investigates the possibility to derive threshold effect levels (TELs) or PNECs from field data. The TELs are termed F-PNECs in this report to differentiate them from PNECs estimated from laboratory experiments, which follow strict calculations rules to derive its value (see Box 1.1, 1.2 and 1.3). A PNEC is defined as the concentration below which unacceptable effects on organisms (i.e. in this report the benthos community on the Norwegian Continental Shelf) will most likely not occur (Anonym 2003).

The purpose of risk assessment is to protect ecosystems from adverse effects. A short outline of the procedure of a risk assessment may be as following: (1) identify the various levels of toxic stressors that the ecosystem is exposed to (e.g. the predicted or measured environmental concentration; PEC or MEC), (2) calculate an effect indicator that describes the relationship between the contaminant levels and their effects on species representing the true ecosystem (e.g. Predicted No Effect Concentration; PNEC) and (3) finally, predict the risk quotient (R) for the ecosystem by comparing the results from step 1 and 2 above, i.e. R = PEC/PNEC. If the risk quotient is less than 1, no adverse effect on the ecosystem would be expected.

The most common method of deriving PNECs is to use assessment factors (safety factors) on single species toxicity effect (response) data generated from laboratory experiments (Box 1.1). The main underlying assumptions of using assessment factors are (Anonym 2003): (1) ecosystem sensitivity depends on the most sensitive species, and (2) protecting ecosystem structure protects community function. These assumptions are necessary because the method discard all results but the most sensitive (Box 1.1). The size of the assessment factor depends on the selection of test species and for how long they have been tested, ranging from chronic (e.g. No Effect Concentration; NOEC) to acute (e.g. Lethal Concentration; LC50). In marine aquatic ecosystem the assessment factors range from 10 to 10.000 (Anonym 2003). The large assessment factors are a result of the huge uncertainties in extrapolating laboratory toxicity test results to actual effect on marine sediment-dwelling species in the nature (e.g. from irrelevant species to relevant species, from a few species to many from species to ecosystems, from laboratory to the field etc.).

A general problem in deriving PNECs of toxic substances in marine sediment is the lack of adequate and relevant data. Normally more data are available for aquatic marine organisms. In the

absence of ecotoxicological data for sediment and sediment-dwelling organisms, the PNECs for marine sediments may provisionally calculated using the equilibrium partitioning method (Box 1.2). The ratio between the concentrations of the substance adsorbed on sediments and dissolved in water (porewater) may be determined by a set of equations for organic substances, and experimentally for metals (Anonym 2003). The main underlying assumptions of using partitioning methods are (Anonym 2003): (1) benthos and planktons organism are equally sensitive for the given substance and (2) the given substance are in thermodynamic equilibrium in organisms, sediments and porewater.

A disadvantage using assessment factors is that it discards a lot of information since it only uses the most sensitive species (see Box 1.1). To utilize all experimental response data one may construct species sensitivity distributions (SSDs) which combines the result from several single species tests with statistical methods (Box 1.3). Various functions may be used to derive SSDs (e.g. Weibull, log-logistic and log-normal). From the SSD a PNEC<sub>x</sub> may be quantified to protect a desired fraction of the "whole" ecosystem (e.g. x = 5 - 100 - 5 = 95 %). The main underlying assumptions of the SSD method are (Anonym 2003): (1) the distribution of species sensitivities follows a theoretical distribution function and (2) the group of species tested in the laboratory is a random sample of this distribution.

As an alternative of deriving PNECs from laboratory eco-toxicity bioassays, this report investigates the possibility to derive F-PNECs from field data of benthic macro benthos living in a gradient of contaminants. The objective is to plot single species abundance data against concentrations to portray species sensitivity. From scatter plots of all sensitive species, effect concentrations (EC50s – see methods) are estimated using regression technique. The EC50s are presently used to construct F-SSDs, from which F-PNECs are derived. The method requirements are large datasets with chemical and biota information for sediment samples ranging from unpolluted to highly polluted; taken from the area that is being studied.

#### **Box 1.1. Assessment factors**

Long term experiments have been performed to derive no effect concentrations (NOECs) for substance x from different saltwater species representing different trophic levels.

Experiment 1: Test species: 1 alga and 1 fish.

The NOEC for the alga was 10 mg/kg and the NOEC for the fish was 5000 mg/kg. Since toxicity data are only available for two trophic levels an assessment factor of 500 is applied to the lowest NOEC, i.e. the PNEC for this "ecosystem" would be: 10/500 = 0.02 mg/kg. If the endpoint for the experiment had been LC50 (i.e. short time experiment), the assessment factor would have been > 10.000, i.e. the PNEC would have been 10.000 times lower than the measured values in the experiment.

Experiment 2: Test species: 1 alga, 4 crustacean, 9 mollusks, 2 sea stars and 4 fishes.

Since more species are tested, the uncertainties are reduced and the magnitude of the assessment factor may be lowered to 10. If the most sensitive species is the same algae as in experiment 1 (i.e. NOEC = 10 mg/kg), the PNEC for the "ecosystem" may now be increased to 10/10 = 1 mg/kg, i.e. 50 times higher than the PNEC for the first experiment.

Strictly theoretically, one may add test organisms infinitively and still only use the result for the algae given that this is a "super sensitive species" for the substance in question.

## **Box 1.2. Equilibrium partitioning theory**

Long term experiments have been performed to derive no effect concentrations (NOECs) for substance x from different saltwater species representing different trophic levels.

Experiment: Test species: 1 alga, 4 crustacean, 9 mollusks, 2 sea stars and 4 fishes.

The PNEC<sub>water</sub> for this "ecosystem" is estimated to be: 10/10 = 1 mg/L (see Box 1.1). This is now an aquatic toxicity test, i.e. using water as the test medium. To derive PNEC<sub>sediment</sub> from PNEC<sub>water</sub>, the following equation is used:

$$PNEC_{sedww} = \frac{K_{susp-water}}{RHO_{susp}} \times PNEC_{water} \times 1000$$
, where

 $K_{susp water}$  is the partition coefficient suspended matter water [m<sup>3</sup>.m<sup>-3</sup>], RHO<sub>susp</sub> is the bulk density of wet suspended matter [kg.m-3]. See equations 18, 22, 23, 24, in TGD document for how to estimate these parameters (Anonym 2003).

Using the formulas and numbers for alkylphenols we have Kp = 150 and RHO = 1000 according to EU-TDG. Thus the PNEC<sub>sediment ww</sub> = 150 mg/kg. Converting to dry weight yields PNEC<sub>sediment dw</sub> =150 \* 4.6 = 690 mg/kg. Note: this value is not comparable to the PNEC in Box 1.1.

#### **Box 1.3. Species Sensitivity Distribution**

Experiment: Long term experiments have been performed to derive no effect concentrations (NOECs) for substance x from different saltwater species representing different trophic levels.

Test species: 1 alga, 4 crustacean, 9 mollusks, 2 sea stars and 4 fishes.

The results are given in the table to the right. The log transformed NOECs (red dots) with SSD (blue line) are shown in the figure. It is assumed that the species sensitivity distributions follow the logistic, S-shaped growth curve (see page 14 for an explanation of the parameters):



Species	logNOEC	PAF
Algae 1	1.00	5
Crustacean 1	1.95	10
Crustacean 2	2.00	15
Crustacean 3	2.04	20
Crustacean 4	2.09	25
Molluse 1	2.19	30
Molluse 2	2.48	35
Molluse 3	2.48	40
Mollusc 4	2.60	45
Molluse 5	2.66	50
Mollusc 6	2.70	55
Mollusc 7	2.70	60
Mollusc 8	2.70	65
Mollusc 9	3.00	70
Sea star1	3.30	75
Sea star 2	3.54	80
Fish 1	3.60	85
Fish 2	3.70	90
Fish 3	4.30	95
Fish 4	4.54	100

Substituting *y* with 5 % in the formula yields  $PNEC_5 = 25 \text{ mg/kg}$ .

$$x = -\frac{\log\left(\frac{5-a}{a \times b}\right)}{c}$$
, where  $a = 95$ ,  $b = 529$  and  $c = 2.42$ ;  $R^2 = 0.97$ .

That is 25 times higher than what we got by using assessments factors. In theory these values should be identical.

One reason for the big difference has to do with the choice of test species. From the NOEC for the alga to the second most sensitive species there is a jump in sensitivity from 10 mg/kg to 90 mg/kg ( $10^{1.95} = 90$ ). However, even if the alga was not included in the experiment, one would get much higher PNECs using F-SSD versus assessment factors (36 mg/kg versus 9 mg/kg).

$$y = \frac{a}{1 + be^{(-cx)}}$$

# 2 The MOD-database

The data are extracted from the Norwegian Oil Industry Association (OLF) database; the MODdatabase. The MOD-database contains biological and chemical information collected from oil monitoring surveys on the Norwegian Continental Shelf in the period 1990 to 2001 (Fig. 2.1). In total 2,206 species and 3,238,371 individuals have been collected in 2,428 sediment samples. The animals are macro benthos (> 1 mm round sieve). All surveys are primarily carried out using the same design and analytic procedures and all participant consultant firms has been accredited according to ISO/IEC/EN 17025 (see Anon 1990, Anon 1999, Anon 2002 and NS 4770 for more detailed description of design and methods). In short: The sampling design is a cross around the oil platforms so that samples are collected from both unaffected zones, intermediate zones and highly affected zones (Fig. 2.1). Pooled abundance data from 5 sediment samples a' 0.1 m<sup>2</sup> make up the abundance at a sampling station. The average concentration of 3 sub-samples (5 at reference and regional stations) taken from the upper sediment layer (0 - 1 cm) make up the concentration at a sampling station. The analytic methods and digestion techniques are believed to derive concentrations that are biological available for sediment dwelling animals (see references above). All concentration are in mg kg<sup>-1</sup>, dry weight (ppm). The surveys are done in regular intervals of minimum 3 years at each region (Fig. 2.1). Thus, several of the sediment samples are collected at the same location (sampling station) at least twice. The average depth is 189 meters ( $\pm$  133 m), ranging from 63 meters to 1500 meters.

#### 3 The datasets

Three dataset were extracted from the MOD-database and F-SSDs were constructed for minimum 11 substances for each dataset. Only dataset 1 and 2 are included in the main report (see Appendix 8.2 for the dataset 3; bottom types). The sensitive animals used to construct F-SSDs were representative for the whole database, except crustaceans that were underrepresented<sup>2</sup>, especially in dataset 1 were only 10 % of the investigated animals were crustaceans, in contrast to 29 % in the whole MOD-database (Fig. 2.1). This difference is due to the fact that crustaceans are found in fewer sediment samples and in lower abundance than the other animal groups and not because

 $<sup>^{2}</sup>$  Traits for most of the > 2000 species are unknown so how representative the investigated species is regarding function, trophic level etc. is unknown.

crustaceans are more tolerant to the investigated contaminants. In total, ca 25 % of the species in the MOD-database has been found in only 1 or 2 sediment samples during this period (1990 - 2001).

**Dataset 1:** To find suitable species to derive F-SSDs, i.e. species occurring in many sediment samples with a wide range of concentrations, the 191 most common species (> 2000 individuals in total) in the MOD-database were extracted. Data collected in the period 1996 to 2001 were further used to derive F-SSD and F-PNECs.

(b)

(a)



Fig. 2. 1: Norwegian continental shelf with the 7 sampling regions, i.e. oil fields (a), sampling design (b) and taxa 1990-2001 (c).

**Dataset 2:** To investigate the influence on grain size on the toxicity threshold values (PNECs), the sediment samples were divided into three datasets with increasing grain size (Fig. 3.1). Due to problems with adequate data for the bottom types (see Appendix 8.2), the dataset consist of all data in the MOD-database (1990 – 2001).

- (1) Dataset 2a: Grain size interval 1:  $< 63 \mu m$
- (2) Dataset 2b: Grain size interval: > 63  $\mu$ m and < 94  $\mu$ m

("mud - silt") ("mud – fine sand") ("fine sand – sand")

(3) Dataset 2c: Grain size interval: > 94  $\mu$ m



Fig. 3.1: Frequency of samples and median grain size. It was logic to put mud (<63) in one category. The rest of the sediment samples were divided in two groups. A finer grain size resolution was not feasible because the need of good resolution on the x-axis in the scatter diagrams (see criteria's on page 12).

F-SSDs and F-PNECs were derived for the following substances:

#### I. Hydrocarbons:

- A. THC
- B. NPD (sum)
- C. PAH (sum of 15 components, measured from 1996 and onwards)
  - 1. PAH1 (1-ring aromatic compound)
  - 2. PAH2 (2-3 ring aromatic compounds)
  - 3. PAH3 (4-6 ring aromatic compounds)
- D. PAHc (PAH<sub>13</sub> and PAH<sub>15</sub> pooled, see comments below).
- E. Decalins (sum of 4 compounds)

#### II. Metals:

- A. Ba
- B. Cd

- C. Cr
- D. Cu
- E. Hg
- F. Pb
- G. Zn

**Comments about PAH:** PAH consist of 13 compounds from 1990 to 1996 and 15 compounds from 1996 and onwards (naphthalene and acenaphthylene is added). PAHc is used as term when PAH<sub>13</sub> and PAH<sub>15</sub> are added together. Adding the two groups together was necessary to get enough data to fulfil the two criteria's regarding abundance and occurrence for all of the three grain size intervals.

For 233 sediment samples<sup>3</sup>, the 15 most important compounds that constitute PAH<sub>15</sub> were divided into the three subgroups: (1) PAH 1, (2) PAH 2 and (3) PAH 3: Note: Benzo (a) fluoranthene and Benzo (k) fluoranthene is pooled to Benzo (b,j,k) fluoranthene. Note: For Vøring 1998 (4 samples), Heidrun Nord 1998 (3 samples), and Finnmark 1998 (3 samples) Phenathrene - Anthracene and Fluoranthene - Pyrene are considered as single compounds. In these cases, both combinations are regarded as belonging to subgroup PAH 2.

PAH 1: Naphthalene	Naphthalene
<b>PAH 2</b> : 2 – 3 rings aromatic compounds	Acenaphthene Acenaphthylene Fluorene Phenanthrene Anthracene
	Fluoranthene
<b>PAH 3</b> : 4 – 6 rings aromatic compounds	Pyrene Benzo(a)anthracene Chrysene/Triphenylene Benzo(a)pyrene Benzo(b,j,k)fluoranthene Benzo(g,h,i)perylene Indeno(1,2,3-c,d)pyren Dibenzo(a,h)anthracene

Single PAH compounds have later been extracted from the MOD-database, but these are not investigated due to time limits of the project. These will however be included in analysis later (in connection with a phd-degree) and the result will be made available when they are finished.

<sup>&</sup>lt;sup>3</sup> Only 233 since this had to be done manually from hardcopies and Excel sheets.

# 4 Methods

**Deriving Effect Concentrations (EC50)**: To investigate species sensitivity, and to estimate effect concentrations (EC50s), scatter plots were constructed for all combinations of species abundance and substance concentrations. Two different extraction methods were tested and evaluated for a sub dataset of 10 species and 4 substances (a pilot early in the process). The methods were (Fig. 4.1.).

- (1) Linear regression model based on average abundance in 10 concentration intervals (Fig. 4.1 a, b).
- (2) Linear and non-linear regression models based on max abundance for continuously unique concentrations levels (Fig. 4. c, d, e, f).

Based on their performances, mainly arbitrary evaluated by information of the species expected sensitivity and mapping their occurrence with GIS-map, the linear regression method performed best and was selected as method (pilot study to decide method to use, presented at Statoil in Trondheim). This means that an effect concentration (EC50) corresponding to 50% reduction in relative abundance was computed using a linear regression model. Although EC10 is closer to the No Observable Effect Concentration (NOEC) and has been used elsewhere in laboratory ecotoxicity tests, a reliable EC10 value cannot be determined from the scatter plot in this study due to large variances at both ends of the regression models. The non-linear model was too restrictive (4.1. c and d) and the reduced matrix scatter gram (4.1 d and e) was too vulnerable for leverage points (a pilot early in the process).

Only species responding positive to a substance (defined as sensitive species) were used to derive EC50s and only if they fulfilled the following two criteria's:

- 1. The species must be occurring in more than 30 sediment samples with different concentration levels, i.e. this set the minimum resolution on the x-axis.
- 2. The abundance of the species must be greater than 100 individuals, i.e. this set the minimum resolution on the y-axis.



**Figure 4.1:** Examples of how the EC50 is obtained for a species based on the pilot study in determine the method to extract EC50s. Each blue point represents the number of relative number of individuals in on sediment sample (sum of 5 grabs), e.g. the 2 in the lower right figure represents 2 % of the maximum number of individuals found in sediment samples with concentrations ranging from around 0 to 3000 mg/kg. First a regression line is applied to fit the relative abundance data (a, c, e) and then an average maximum is used as the EC100 (intercept) to compute the EC50 (b,d,f). In (a) and (b) the scatter plot is reduced to a 10 x 10 point matrices by calculating the average abundance in 10 intervals before the regression line is estimated.

For dataset 2, a criterion about concentration range was set in order to ensure sufficient range on the x-axis (see Appendix 8.2): The upper concentration range must be 1.5 times the F-PNECs derived from dataset 1. For example: 800 individuals of species Y have been found in 100 samples with concentration ranging from 1 to 2 mg/kg. The predicted effect concentration for the substance was however estimated to 15 mg/kg in the first analysis (dataset 1). Thus, the scatter plot gives no information about the abundance at concentrations were an effect would be expected (in this example the upper concentration range had to be 15\*1.5 = 23). See also Appendix 8.2; bottom types.

**Deriving F-SSD:** Based on the criteria's above on average 113 sensitive species, ranging from 22 for PAH to 200 for THC, were used to construct F-SSDs for dataset 1 and 2 (Table 4.1). Two methods were used to construct F-SSDs:

- (1) Sigmoid model
- (2) Bootstrapping

The sigmoid model assumes that the distribution of species sensitivity follows an S-shaped growth curve. The selected curve is a reparameterised version of the logistic equation and looks like this:

$$y = \frac{a}{1 + be^{(-cx)}} \tag{1}$$

where x = concentrations (here log) and y = cumulative affected fraction of species. The other letters are parameters: a is the asymptotic value of the cumulative affected fractions of species, b is related to the intercept and c is the exponential increase in sensitivity at small concentrations. Since only sensitive species are use to construct F-SSDs, the cumulative potentially affected fraction of species are allowed to be below 100 % (i.e. unconstrained "a"; see also formulae 3). Since the essential protection levels are at the lower tail of the curve, the influence of a low "a" parameter has little influence on the resulting threshold effect levels (F-PNECs). Note however that the sigmoid model is unconventional in respect to SSDs.

The bootstrap method obtains a point estimate (referred to as the bootstrap estimate) together with such a range for the estimate (referred to as a bootstrap confidence interval) by focusing on the set of values taken by the estimate over many resample of size n drawn randomly from the original sample with replacement (Grist, et al. 2002). The bootstrapping techniques do not make any

Substances	Dataset 1	Dataset 2 a	Dataset 2b	Dataset 2c
Substances	(common species)	("mud")	("mud – fine sand")	("fine sand – sand")
Ba	73	170	185	165
Cd	105	97	182	164
Cr	110	124	39	70
Cu	116	146	191	191
Hg	94	58	80	NA
Pb	108	146	195	165
Zn	111	141	171	174
ТНС	128	142	200	181
NPD	96	58	85	65
РАН	100	NA	22	26
РАНс	NI	51	76	69
1 ring PAH	79	NA	NA	NA
2-3 ring PAH	81	NA	NA	NA
4-6 ring PAH	94	NA	NA	NA
Decalins	135	41	47	61

 Table 4.1: Number of sensitive species used to construct F-SSDs for Dataset 1 and Dataset 2.

NI means that the substance has not been investigated and NA means that there were not adequate data to derive F-SSDs (see criteria's in Methods).

distributional assumptions since one draw data repeatedly and random from the EC50-pool. This gives the possibility to predict confidence interval for the whole SSD range. To reduce (stabilize) the random sampling error, data were re-sampled 5000 times (B = 5000).

**Deriving PNEC:** Two F-PNEC-values (F-PNEC<sub>5</sub> and F-PNEC<sub>x</sub>) were estimated from the F-SSDs: The first F-PNEC was determined as the  $5^{th}$  percentile protection level by substituting 5 % for *y* in the logistic equation:

$$PNEC = \ln \frac{\left[\frac{a/y}{b} - 1\right]}{c}$$
(2)

For the bootstrapping technique the PNECs was read directly off the resulting data matrices. The second F-PNEC was determined as the  $y_1$ <sup>th</sup> percentile protection level by taking into consideration the species regarded as not sensitive:

$$y_1 = \frac{y (i.e. 5\%)}{N_{SS} / (N_{IS} - N_{RS})}$$
(3)

where NSS is the number of sensitive species, NIS is the number of investigated species and NRS is the number of rare species which is defined as the species not fulfilling criteria 1 or criteria 2 listed above.

# **5** Results

**Part I:** Figure 5.1 shows the two F-SSD types for Ba and Cd derived from the 191 most common species in MOD. F-SSDs for the other substances are given in Appendix 1. As seen from the F-SDDS, Ba is not very toxic to sediment-dwelling macro benthos (F-PNEC = 2283 mg/kg), while Cd is regarded as more toxic (F-PNEC = 0.046 mg/kg). All F-PNECs derived from F-SSDs using the bootstrap technique is given in Table 5.1, and F-PNECs derived from F-SSDs using the logistic model are given in Table 5.2.



Fig. 5.1: Field based species sensitivity distribution for Ba and Cd. (a) F-SSD for Ba, using bootstrap (b) F-SSD for Ba, using a logistic function. (c) F-SSD for Cd using bootstrap. (d) F-SSD for Cd, using a logistic function. The solid line in (a) and (c) shows the median estimate (50%) percentile with lower (5%) and upper (95%) percentiles (dashed lines), which constitutes a 90% two-tailed percentile confidence interval. The solid line in (c) and (d) shows the logistic curve.

Substances	F-PNEC <sub>5</sub>	95 % - low	95 % - high	F-PNEC <sub>x</sub>	95 % - low	95 % - high	X
Substances	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(%)
Ba	1718	1644	2020	2283	1938	2522	13
Cd	0.039	0.036	0.046	0.046	0.040	0.050	9
Cr	9.801	7.795	10.965	10.763	9.837	11.909	8
Cu	6.438	5.166	7.568	7.377	6.464	8.189	8
Hg	0.023	0.019	0.026	0.026	0.025	0.030	10
Pb	11.50	10.44	12.83	12.83	11.80	16.57	9
Zn	26.59	22.81	31.95	31.07	26.62	37.98	8
ТНС	100.2	82.85	126.1	123.5	96.48	131.0	7
NPD (sum)	0.205	0.166	0.276	0.273	0.223	0.357	9
PAH (sum)	0.075	0.074	0.115	0.099	0.075	0.154	8
Decalins	8.810	3.333	13.20	10.17	7.460	19.17	7

**Table 5.1:** F-PNECs for the 191 most common species in MOD (dataset 1) derived from F-SSDs using bootstrapping: Medians and 95% confidence intervals for F-PNECs at the apparent 5 % protection level (F-PNEC<sub>5</sub>) and for the adjusted 5 % protection level (F-PNECx).

**Table 5.2:** F-PNECs for the 191 most common species in MOD derived from F-SSDs using logistic function F-PNECs at the apparent 5 % protection level (F-PNEC5) and for the adjusted 5 % protection level (F-PNECx).

Substances	F-PNEC <sub>5</sub>	F-PNEC <sub>x</sub>	X
Substances	(mg/kg)	(mg/kg)	(%)
Ba	1148	1793	13
Cd	0.035	0.042	9
Cr	7.527	9.340	8
Cu	4.361	5.620	8
Hg	0.022	0.023	10
Pb	10.36	12.84	9
Zn	21.96	27.95	8
ТНС	56.41	79.57	7
NPD (sum)	0.196	0.285	9
PAH (sum)	0.084	0.111	8
Naphthalene	0.005	0.006	9
2-3 ring PAH*	0.022	0.032	9
4-6 ring PAH*	0.073	0.095	8
Decalins	7.860	9.82	7

**Part II:** Figure 5.2 illustrates the two F-SSD types for Ba and Cd derived from dataset 2; i.e. (2a) grain size interval 1 ("mud"), (2b) grain size interval 2 ("mud – fine sand"), and (2c) grain size interval 3 ("fine sand – sand"). F-SSDs for all substances are given in Appendix 1. The F-PNECs derived from bootstrapping are given in Table 5.3, 5.4 and 5.5, and F-PNECs derived from the logistic function are given in Table 5.6. As previously, Ba is not very toxic to sediment-dwelling macro benthos (F-PNEC = 2645 mg/kg, 2263 mg/kg and 1951 mg/kg), while Cd is regarded as more toxic (F-PNEC = 0.052 mg/kg, 0.046 mg/kg, and 0.089 mg/kg). Note the poor fit at the 0 -



**Fig. 5.2**: Field based species sensitivity distribution for Ba and Cd using basic bootstrap method. (a) Ba and grain size interval 1 (b) Ba and grain size interval 2 (c) Ba and grain size interval 3 (d) Cd and grain size interval 1 (e) Cd and grain size interval 2 (f) Cd and grain size interval 3. The solid line is median estimate (50%) percentile with lower (5%) and upper (95%) percentiles (dashed lines), which constitutes a 90% two-tailed percentile confidence interval.



**Fig. 5.3**: Field based species sensitivity distribution for Ba and Cd using logistic function: (a) Ba and grain size interval 1 (b) Ba and grain size interval 2 (c) Ba and grain size interval 3 (d) Cd and grain size interval 1 (e) Cd and grain size interval 2 (f) Cd and grain size interval 3.

Substances	F-PNEC <sub>5</sub>	95 % - low	95 % - high	F-PNEC <sub>x</sub>	95 % - low	95 % - high	X
Substances	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(%)
Ba	1977	1808	2275	2645	2409	3181	13
Cd	0.048	0.037	0.052	0.052	0.048	0.055	9
Cr	13.011	11.581	14.245	14.14	12.389	15.411	7
Cu	6.867	6.302	7.321	7.374	7.083	7.982	9
Hg	0.030	0.028	0.035	0.034	0.030	0.040	9
Pb	16.60	13.98	18.09	18.65	17.11	20.56	9
Zn	30.87	29.91	33.57	34.00	30.89	36.53	9
ТНС	55.8	42.91	65.4	71.2	60.02	81.7	9
NPD (sum)	0.212	0.190	0.255	0.244	0.216	0.354	9
PAH (sum)	0.177	0.177	0.223	0.187	0.178	0.223	10
PAHc (sum)	0.187	0.164	0.232	0.238	0.194	0.266	10
Decalins	8.471	7.678	13.27	10.45	8.300	15.92	8

**Table 5.3:** F-PNECs for dataset 2a ("mud") derived from F-SSDs using bootstrapping. Medians and 95% confidence intervals for F-PNECs at the apparent 5 % protection level and for the adjusted F-PNECs; F-PNECx i.e. the actual concentration level below which harmful effects on the benthic community are unlikely to be observed.

**Table 5.4:** F-PNECs for dataset 2b ("mud – fine sand") derived from F-SSDs using bootstrapping. Medians and 95% confidence intervals for F-PNECs at the apparent 5 % protection level and for the adjusted F-PNECs; F-PNECx i.e. the concentration level below which harmful effects on the benthic community are unlikely to be observed.

Substances	F-PNEC <sub>5</sub>	95 % - low	95 % - high	F-PNEC <sub>x</sub>	95 % - low	95 % - high	X
Substances	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(%)
Ba	1720	1372	2200	2263	2141	2490	10
Cd	0.041	0.032	0.046	0.046	0.041	0.052	8
Cr	7.319	5.128	8.659	7.901	6.845	10.835	8
Cu	5.111	4.793	5.652	5.725	5.562	6.509	9
Hg	0.111	0.088	0.197	0.174	0.101	0.303	7
Pb	10.57	10.18	12.04	12.76	11.52	15.00	9
Zn	22.53	20.62	27.75	30.42	26.58	33.25	10
ТНС	98.5	89.52	125.9	130.5	100.6	148.6	8
NPD (sum)	0.181	0.149	0.212	0.217	0.191	0.256	10
PAH (sum)	NA	NA	NA	NA	NA	NA	NA
PAHc (sum)	0.153	0.130	0.174	0.191	0.158	0.202	10
Decalins	17.983	12.676	20.11	20.22	19.377	22.43	11

 $15^{\text{th}}$  percentile for Ba and Cd in the logistic F-SSDs (Fig. 5.1 a, Fig. 5.3 d, e and f). The logistic F-SSDs predict lower F-PNECs than the EC50s indicates i.e. the F-SSDs are shifted to the left of the estimated effect values (red dots). Poor fit at the endpoints were common in several of the investigated substances (see Appendix 1). For the bootstrap F-SSDs, the complexity at the endpoint is reflected in the confidence intervals, e.g. there is an increase in the 95 % confidence intervals for Cd in Fig. 5.3 a, b, c (c.i. = 0.07, 0.11 and 0.36 mg/kg, respectively), correlated to the increasing

Substances	F-PNEC <sub>5</sub>	95 % - low	95 % - high	F-PNEC <sub>x</sub>	95 % - low	95 % - high	x
Substances	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(%)
Ba	1711	1498	1929	1951	1816	2254	9
Cd	0.074	0.059	0.096	0.089	0.062	0.098	6
Cr	7.184	6.869	8.216	8.211	7.273	9.031	9
Cu	5.024	4.831	5.639	6.291	5.419	7.856	9
Hg	NA	NA	NA	NA	NA	NA	NA
Pb	10.66	9.48	12.59	12.55	10.90	13.71	8
Zn	25.34	22.03	28.55	28.49	25.39	35.48	8
ТНС	70.0	65.59	97.9	99.1	70.72	123.1	8
NPD (sum)	0.459	0.403	0.564	0.480	0.420	0.595	6
PAH (sum)	0.108	0.102	0.149	0.129	0.110	0.179	9
PAHc (sum)	0.078	0.071	0.098	0.094	0.080	0.138	9
Decalins	15.53	14.851	18.33	16.34	15.470	21.36	8

**Table 5.5:** F-PNECs for dataset 2c ("fine sand –sand") derived from F-SSDs using bootstrapping. Medians and 95% confidence intervals for F-PNECs at the apparent 5 % protection level and for the adjusted F-PNECs; F-PNECx i.e. the actual concentration level below which harmful effect on the benthic community are unlikely to be observed.

**Table 5.6:** F-PNECs for dataset 2 a ("mud"), b ("mud – fine sand"), c ("fine sand – sand") derived from F-SSDs using logistic function. Only the adjusted F-PNECs (F-PNECx) are given, i.e. the actual concentration levels below which harmful effect on the benthic community are unlikely to be observed.

Substances	F-PNEC <sub>x</sub> ("mud")	X	F-PNEC <sub>x</sub> ("mud – fine sand")	X	F-PNEC <sub>x</sub> ("fine sand – sand")	X
	(mg/kg)	%	(mg/kg)	%	(mg/kg)	%
Ba	2200	13	1931	10	1942	9
Cd	0.042	9	0.031	8	0.050	6
Cr	7.400	7	9.116	8	4.836	9
Cu	6.587	9	4.877	9	4.167	9
Hg	0.026	9	0.937	7	NA	NA
Pb	16.15	9	11.68	9	10.60	8
Zn	29.43	9	25.07	10	23.93	8
ТНС	41.95	9	99.62	8	72.82	8
NPD (sum)	0.231	9	0.144	10	0.343	6
PAH (sum)	0.146	10	NA	NA	0.134	9
PAHc (sum)*	0.196	10	0.108	10	0.097	9
Decalins	8.336	8	16.98	11	12.33	8

in the logistic F-SSDs (i.e. poorer and poorer fit) for Cd towards the left (Fig. 5.3 d, e and f). Note also that the F-SSDs evens out before it reach 100 % affected fraction of species (e.g. Fig. 5.3 e). This is because it is not put any constrained for the asymptotic value a, which logical should be set to 100. Although this has little practical effect at lower protection level, the result from the bootstrapping is regarded as more valid.

In Table 5.7, F-PNECs estimated from F-SSDs derived using the bootstrap technique are compared with F-PNECs estimated from F-SSDs derived using the logistic equation. The F-PNECs estimated from bootstrap F-SSDs are on average 23 %  $\pm$  29 % higher than the F-PNECs estimated from logistic F-SSDs. For example, continuing the example above, the bootstrap F-PNEC for Cd is 0.046/0.042 = 10 % higher than the logistic F-PNEC for dataset 1 (Fig. 5.1 c and d), which has an almost perfect fit at the endpoint (Fig. 5.1 d), and for dataset 2a, b and c which has increasingly poorer fit (Fig. 5.3 d, e and f), the difference is 24 %, 48 % and 78 %, respectively.

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Substances	Dataset 1 (common species)	Dataset 2 a ("mud")	Dataset 2b ("mud – fine sand")	Dataset 2c ("fine sand – sand")
Ba	1.27	1.20	1.17	1.00
Cd	1.10	1.24	1.48	1.78
Cr	1.15	1.91	0.87	1.70
Cu	1.31	1.12	1.17	1.51
Hg	1.13	1.31	0.19	NA
Pb	1.00	1.15	1.09	1.18
Zn	1.11	1.16	1.21	1.19
ТНС	1.55	1.70	1.31	1.36
NPD (sum)	0.96	1.06	1.51	1.40
PAH (sum)	0.89	1.28	NA	0.96
PAHc (sum)	NA	1.21	1.77	0.97
Decalins	1.04	1.25	1.19	1.33
Average	1.14	1.30	1.18	1.31
SD	0.19	0.25	0.41	0.28

 Table 5.7: Comparison of adjusted F-PNECs derived from bootstrap and logistic F-SSDs (PNEC<sub>boostrap</sub>/PNEC<sub>logistic</sub>.)

There were no consistent differences of the PNECs, for the three grain size intervals, derived from F-SSD using bootstrap method (Figure 5.4). In total, 73 % of the substance had higher F-PNECs for G1 than G2 (grain size interval 1 and 2), 64 % higher F-PNECs for G1 than G3, and 60 % had higher F-PNECs for G2 than G3 (grain size interval 3). Thus, although there is a small tendency of decreasing F-PNEC with increasing grain size, it is not the clear pattern that might be expected (Pesch, 1979; Swartz et al., 1985; Kemp and Swartz, 1988; Bryan and Langston, 1992; Green et al., 1993; Trannum et al., 2004), i.e. F-PNEC<sub>G1</sub> > F-PNEC<sub>G2</sub> > F-PNEC<sub>G3</sub>.

One possible reason for a missing pattern in the grain size F-PNECs may be that the method is not able to detect the differences, e.g. because the method is not sensitive enough or that there exist unmeasured factors (i.e. factors not available for this project) such as salinity and pH etc. that

compensate (i.e. inverse relationship) for the expected increasing toxicity with increasing grain size. Moreover, although not an obvious relation, it has been shown that the species richness in benthic communities on the Norwegian Continental Shelf increase with increasing grain size (Frode Olsgard, NIVA, personal communication). Another plausible explanation is that there is overlap between the selected grain size intervals. As seen in Figure 3.2, a greater part of the sediment samples have median grain size of 80 to 100  $\mu$ m, and this was divided between Dataset 2b and 2c. Thus, although the SSDs take the whole species sensitivity distribution into account, the most important species determining the community overall sensitivity are still the highly sensitive species (see also Box 1.1. and 1.3). Moreover, since median diameter are used as a measurement of grain size in the MOD-database, a sediment sample belonging to e.g. grain size interval 2 will usually also consist of grains with sizes less than 63  $\mu$ m (e.g. sediment sample no. 07 from the Veslefrikk oil platform in 2001 has a median grain size diameter of 93  $\mu$ m, i.e. it is located in the upper limit of grain size interval 2 and 3, consist also of 16.3 % particles with diameter smaller than 63  $\mu$ m).



**Fig. 5.4:** Comparison of adjusted F-PNECs derived from F-SSD using bootstrap method: G1= Dataset 2 a ("mud"), G2 = Dataset 2b ("mud - fine sand"), and G3 = Dataset 2c ("fine sand – sand"). The data are normalized for F-PNEC for Ba in Dataset 2a (F-PNECx = 2645 mg/kg). Note the scale: 1 - 100.000.

In Table 5.8 the F-PNECs are compared with the overall background concentrations and Limit of Significance Contamination; LSC (see Appendix 2 for an interpretation of LSC). The F-PNECs are

	Dataset 1	Dataset 2a	Dataset 2b	Dataset 2c	Mean Dataset 2	Background*	LSC**
Substances	F-PNEC <sub>x</sub>	F-PNEC <sub>x</sub>	PNEC <sub>x</sub>	F-PNEC <sub>x</sub>	F-PNEC <sub>x</sub>	(mg/kg)	(mg/kg)
Ba	2283	2645	2263	1951	2286	131.5	8.60 - 554
Cd	0.046	0.052	0.046	0.089	0.062	0.037	0.01-0.23
Cr	10.763	14.14	7.901	8.211	10.08	14.62	6.50 - 52.0
Cu	7.377	7.374	5.725	6.291	6.463	4.099	1.00 - 19.0
Hg	0.026	0.034	0.174	NA	0.104	0.021	0.01 - 0.13
Pb	12.83	18.65	12.76	12.55	14.65	10.75	5.30 - 47.9
Zn	31.07	34.00	30.42	28.49	30.97	20.67	6.20 - 94.5
ТНС	123.5	71.2	130.5	99.1	100.3	29.85	5.10 - 35.1
NPD (sum)	0.273	0.244	0.217	0.48	0.183	0.067	0.02 - 0.20
PAH (sum)	0.099	0.187	NA	0.129	0.158	0.088	0.03 - 0.79
PAHc (sum)	NA	0.238	0.191	0.094	0.174	0.174	NA
Decalins	10.17	10.45	20.22	16.34	15.67	0.042	> 0.050***

Table 5.8: Comparison of adjusted F-PNECs derived from bootstrap F-SSDs with overal	l background concentration
and Limit of Significance Contamination; LSC. Values in mg kg <sup>-1</sup> dry weight.	

\* Concentration for all reference and regional stations regardless of grain size intervals.

\*\* Concentrations for reference and regional stations taken into consideration depth, grain size and fauna.

\*\*\* Background concentration and LSC for decalins is set equal to the limit of detection.

on average 5.4 times higher than the background concentration4 (exclusive decalins which are 200 to 400 times higher than the background level), and are located within the LSC-range (Cr, Cu, Pb and Zn), or above the LSC-range (Ba, Cd, Hg, THC, NPD and PAH). Thus, although the F-SSDs are more restrictive than current sediment quality guidelines (see Discussion), they are much higher than the background concentration.

<sup>&</sup>lt;sup>4</sup> Natural background concentrations vary on the Norwegian Continental Shelf and the single values in Table 5.8 and in the text are used to put the F-PNECs in a perspective.

# 6 Discussion and conclusion

EC50: F-SSDs were constructed based on extracting effect data from scatter plots for a large number of species living in a gradient of contaminants. EC50 was used as effect (response) concentration, i.e. the concentration where the abundance decreases by 50 % from abundance found at the background concentration. Two methods were run on a sub dataset; one that tries to portray all variation in abundance by segmenting the distribution of abundance in subsets (Fig. 4.2 a, b) and one that tries to portray the variation in abundance on changes in the means (Fig. 4.2 c, d, e and f). Although the first method provides a more complete view of the effects of the contaminant, the method was susceptible for extreme points in both the response direction (outliers) and in the covariate space (leverage points) and could not be used on the entire dataset. The second method uses the lower part of the scatter plots (mean maximum abundance is set as EC100), and exclude much of the large variation in the natural undisturbed sediment. However, as concentration increases, sensitive species (as defined here) decrease in abundance and the scatter becomes less as one single dominant factor acts (Fig. 4). Thus, the apparently large variation in Fig. 4 a and b and relatively poor 'fit' to the regression lines is probably an accurate representation of the response of the species in the field (Leung, et al. 2005). Since mean maximum is evaluated from changes in abundance at the center and not near the maxima, it may be argued that the response data are actually closer to EC25 than EC50 (Fig 4.2.b versus Fig. 4 d and f). A paper is under preparation that investigates changes in abundance near the maxima, rather than at the center of the response distribution and also utilizing the advantage of bootstrap techniques to avoid the "one size fits all approach" (see also the discussion about F-SSDs below).

**F-SSD:** All F-SSD-regressions were significant and had a coefficient of determination ( $\mathbb{R}^2$ ) of around 0.99. However, several of the logistic F-SSDs portrayed too low values at very low and very high protection levels, compared with the estimated EC50s (e.g. Fig. 5.1 b; see also the figures in Appendix 1). Since no constrain were put on the asymptotic value, the F-SSDs do not flatten out at 100 % (i.e. parameter "*a*" should had been constrained to 100 since this is the final end point). The unconventional log-logistic function was chosen to describe species sensitivity because, in addition to relatively good fit to the effect data, it is a well known distribution in biology studies relating to population growth, decline etc. Several other distributions have been proposed (e.g. Weibull and lognormal) but neither these distributions are necessarily based on especially good ecological or statistical grounds (Wheeler et al. 2002). A general statistical problem using theoretical distributions

is that one assumes that one model will fit all available data. The bootstrap method is an alternative to construct SSDs (Grist, et al. 2002). This method does not require any assumptions about a specific distribution since one draw data randomly directly from the EC50-pool. The bootstrap method do however require more data (> 20 effect data points in order to be able to predict a protection level of 5 %, and preferably > 40) than the standard SSD method. Both methods require that the empirical distribution function obtained from the sample is a reasonable estimate of the cumulative distribution function underlying the true distribution of the population (Grist, et al. 2002). On average the bootstrap F-SSDs yielded 23 % ( $\pm$  29 %) higher protection level than the logistic F-SSDs (Table 5.8). A great deal of this difference is due to poor fit of the logistic function at low protection levels, plausible underestimating the F-PNECs. For example, as seen in Figure 3.1a, the logistic model for Ba yields lower values than indicated by the EC50s at the 0 -  $20^{\text{th}}$ percentile. In contrast, for Cd the logistic model fit very well at the corrected protection level (Fig. 5.1c), and F-PNEC<sub>9</sub> is located within the 95 % confidence interval (Table 5.1 and Table 5.2). In general, even if the fit of the logistic model is very good around the desired protection level, the bootstrap method yielded in general somewhat higher median PNECs, although as illustrated above, it is not any statistical differences (Table 5.8; see also Table 1 in Grist, et al. 2002). In conclusion, the results from the bootstrap F-SSDs are regarded as most valid.

**Comparison:** Due to great uncertainties in deriving PNECs (see Box 1.1., 1.2. and 1.3) it is essential to evaluate several alternative methods in RISK assessment. This is especially important where adverse biological effects are not obviously observable, as is the case on the greater part of the Norwegian Continental Shelf. Within the ERMS project three techniques have been used to derive PNECs. In general, all three methods yielded threshold effect levels (TEL)<sup>5</sup> of similar magnitude of order, with some exceptions, e.g. Hg and decalins (Table 6.1, Table 6.2 and Fig. 6.1, Fig. 6.2). Compared with existing sediment quality guidelines (SQG), the TELs derived within the ERMS project is generally lower, i.e. more restrictive (Fig. 6.1, Fig. 6.2). Most of the current SQGs are derived for coastal species and laboratory species, whereas the Must as and UoO approach is derived from much deeper waters with very different environmental conditions and species fauna. Moreover, the UoO TELs, and partly the Must NOECs take into account possible interacting and synergetic effects. An increase in one substance may alter the effect of other substances since in the field different contaminants will probably interact. A recent study, for example, has demonstrated that, in the presence of PAHs, metal PNECs are abnormally low (Peter Chapman, Golder

<sup>&</sup>lt;sup>5</sup> This report use the adjusted F-PNEC<sub>x</sub> as TEL, Must use NOECs as TEL and TNO use PNEC<sub>5</sub> as TEL.

Associates, Canada; personal communication in Leung et al, 2005). Also nontoxic metals such as Fe may affect the toxicity of resident sediment metals (Rule and Alden, 1996). Similar, the F-PNECs show that if e.g. Cd concentrations in the sediment on the Norwegian Continental Shelf exceeds on average 0.058 mg/kg there will be adverse effect on the benthic community, given that the other substances are present. Thus, if PAHs are removed or lowered, the effect of Cd (and other metals) will probably decrease, and the F-PNECs will increase.

Table 6.1 shows PNECs derived by using a modification of the equilibrium partitioning theory, EqP taking the background concentration into consideration (Anon 2005; see also Box 1.2). Using the background concentration at Haltenbanken, the PNECs (MPC-sediment) are on average  $3.1 \pm 2.9$  times higher than the average F-PNECs derived in this report, ranging from 0.8 for Cd to 8.5 for Hg. The PNECs (MPA-sediment; i.e. the maximum concentration "allowed" to be added to the sediment) for Cu, and Zn is very close to the background concentration; 1% and 1 %, respectively. Thus, using another background concentration than Anon (2005) will change the PNECs, e.g. using the overall background concentration on the Norwegian Continental Shelf, 1990 – 2001 (Table 5.8), the PNECs is on average  $2.5 \pm 3.1$  times higher than the F-PNECs derived in this report, ranging from 0.6 for Cu to 8.6 for Hg<sup>6</sup>.

Metals	PNEC <sub>water</sub> (MPA <sub>water</sub> )*	K <sub>p (barite-water)</sub> sediment	$\frac{\text{PNEC}_{\text{sediment}}}{(\text{MPA}_{\text{sed}})}$	PNEC <sub>sediment</sub> Cb (MPCs <sub>edimen</sub> t)**	PNEC <sub>sediment</sub> (MPC <sub>sed)***</sub>
_	(ug/l)	(L/kg)	(mg/kg)	mg/kg	(mg/kg)
Cd	0.34	28.8	0.0098	0.050	0.050
Cr	8.5	1738	14.77	42.77	29.37
Cu	1.1	43.7	0.048	9.048	4.150
Pb	11	79.4	0.873	17.87	11.57
Hg (methyl)	0.01	61660	0.617	0.627	0.640
Hg****	0.23	61660	14.18	14.19	14.20
Zn	6.6	69.2	0.457	50.46	21.16

Table 6.1: TELs for sediment dwelling benthos living in the Haltenbanken region, and on the Norwegian Continental Shelf derived by equilibrium partitioning method (modified from Anon., 2005). PNEC sediment in mg/kg dry weight.

\* PNEC<sub>water</sub> and MPA<sub>water</sub> is based on statistical extrapolation of aquatic data applied in the Netherlands (Anon., 2005). \*\* MPA-sediment + Haltenbanken background concentration.

\*\*\*MPA-sediment + Norwegian Continental Shelf background concentration. from Table 5.8.

\*\*\*\* Based on total concentration of Hg. Also used as background concentration for Hg methyl.

Table 6.2 shows F-PNECs by Must derived by using multivariate techniques on the same field data (dataset 2) as this report (Grung, et al 2005). Must has used a finer grain size resolution than this

<sup>&</sup>lt;sup>6</sup> This section is updated 29.05.06 based on new Kp values and the point of very low MPC values are not so crucial anymore, although still valid for Cu and Zn.

$\mu$ in, and $\geq$ 94 $\mu$	$\mu$ m, and $\sim 94$ $\mu$ m, respectively. Oram size intervals is in $\mu$ m (e.g. 0.410 $\mu$ m) and the values is in mg/kg dry weight.									
Substances	Dataset 2a	Dataset 2b	Dataset 2c	0-10	10-30	30-50	50-70	70-90	90-110	
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	
Ba	2645	2263	1951	1520	2010	921.0	597.0	532.0	690.0	
Cd	0.052	0.046	0.089	0.106	0.057	0.043	0.021	0.020	0.020	
Cr	14.14	7.901	8.211	33.80	23.90	9.040	5.700	5.570	5.433	
Cu	7.374	5.725	6.291	11.06	7.250	3.800	2.237	1.600	1.170	
Hg	0.034	0.174	NA	0.050	0.020	0.014	0.013	0.010	0.010	
Pb	18.65	12.76	12.55	21.50	18.30	12.90	6.000	7.800	6.470	
Zn	34.00	30.42	28.49	67.90	44.40	17.94	11.82	9.77	6.800	
ТНС	71.20	130.5	<i>99.10</i>	21.40	8.867	9.733	9.400	8.11	8.000	
NPD (sum)	0.244	0.217	0.089	0.093	0.061	0.037	0.035	0.014	0.011	
PAH (sum)	0.187	NA	0.129	0.110	0.07	NA	NA	0.032	0.0088	
PAHc (sum)	0.238	0.191	0.094	NA	NA	NA	NA	NA	NA	
Decalins	10.45	20.22	16.34	0.026	0.021	0.084	0.032	0.036	0.058	

Table 6.2: TELs for sediment dwelling benthos living on the Norwegian Continental Shelf, derived from field data (this report and Grung, et al. 2005).\* Dataset 2a, b and c is sediment samples with median grain size  $< 63 \mu m$ ,  $> 63 and < 94 \mu m$ , and  $> 94 \mu m$ , respectively. Grain size intervals is in  $\mu m$  (e.g. 0 -10  $\mu m$ ) and the values is in mg/kg dry weight

\*This report uses the adjusted F-PNEC<sub>x</sub> as TEL, Grung et. al. (2005) uses NOECs as TEL. The TELS compared in the text are marked with same font - common font (black) bold (red) and italic (blue)

report, especially for mud (< 63  $\mu$ m), which is divided into 3-4 groups. Thus, for comparison, the TELs are grouped into three intervals (Table 6.2). TELs for metals in "mud" derived in this report are on average twice as high as Must TELs (Table 6.2). However, as seen in Table 6.2 and Fig. 6.1, the TELs from Must are in general higher for metals in mud than the TELs derived in this report (e.g. excluding Hg the ratio TEL<sub>UoO</sub>/TEL<sub>Must</sub> is 0.97, and excluding Ba the ratio is 0.76). For the two other grain size intervals the TELs derived in this report are on average 5.1 (ranging from 1.4 for Cr to 17.4 for Hg) and 3.2 (ranging from 1.5 for Cr to 5.4 for Cu; TEL for Hg is not available in this interval) higher than the TELs from Must (see Table 6.2 and Fig. 6.1). The TELs for hydrocarbons derived in this report in "mud" are on average 7.2 times higher than Must TELs; 10.6 for THC and 3.8 for NPD. For the two other grain size intervals, the average ratio is 15.6 and 11.7, respectively (see Table 6.a and Fig. 6.2).

In contrast to this report, the Must TELs show a clear pattern of decreasing TELs at increasing grain size (Table 6.2). The clearest pattern is within the mud intervals (grain size 0 - 70), and except for Cu, NPD and PAH there is no pattern of decreasing TELs for largest particle sizes, i.e. from 50-70, 70-90, and 90-110 µm (Table 6.2). Some of the possible reason for the little effect of grain size in this report is discussed above (see Result). In addition the Must result may indicate that there is a large decrease in sensitivity within Dataset 2 a (< 63 mm) that is not detected in this report.





(b) Cd



(c) Cr





(e) Hg



(f) Pb





Fig. 6.1: Comparison of SQGs in terms of TELs for metals: (a) Ba, (b) Cd, (c) Cr, (d) Cu, (e) Hg, (f) Pb and (g) Zn with Must as (Grung, et al 2005), TNO (Anon, 2005; tno.1 is for Haltenbanken and tno.2 is for the Norwegian Continental Shelf, see Table 6.1) with those derived in the present report. In addition, for some substances, TELs are compared with SQGs from USA (MacDonald, 1994), Norway (ICES, 2003) and Sweden (ICES, 2003). The green area is the overall background concentration, and the red dotted lines are the lower and upper LSC.



b) PAH \* the PAHs are not equal, i.e. consist of different number of components (see also PAH comments in methods)





#### (d) Decalins



Fig. 6.2: Comparison of SQGs in terms of TELs for hydrocarbons: (a) THC, (b) PAH, (c) NPD, and (d) Decalins with Must as (Grung, et al 2005), TNO (Anon, 2005; tho 1 is for Haltenbanken and tho.2 is for the Norwegian Continental Shelf, see Table 6.1) with those derived in the present report. In addition, for PAH, TELs are compared with SQGs from USA (MacDonald, 1994), Norway (ICES, 2003) and Sweden (ICES, 2003). The green area is the overall background concentration, and the red dotted lines are the lower and upper LSC, respectively.

**Conclusion:** The F-SSDs show the response of the sensitive species to the named contaminant in the presence of other contaminants (both measured and not measured). Thus, the F-SSDs account for possible interacting and synergetic effects. Especially the latter may explain some of the restrictive (low) F-PNECs for metals and PAH. In addition, competition and predation are included in the F-SSDs. The F-PNECs may be integrated with current SQG and should be a valuable contribution to existing SQG due to it's ecologically relevance. However, the effect of single substances are not singled out so the normal procedure used to map the stress of several substances, i.e. the combined stress is not appropriate since this will result in "double-entry book-keeping" For the ERMS-project the F-PNECs should be applied for validation purposes, and not as input for a model.

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# 8 Appendixes

8.1 F-SSDs8.2: Bottom types.8.3 Limit of Significant Contamination'8.4 Abbreviations

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# 8.1 F-SSDs

- Figure 8.1: F-SSDs for dataset 1 (common species) using bootstrap method.
- Figure 8.2: F-SSDs for dataset 1 (common species) using logistic function.
- Figure 8.3: F-SSDs for dataset 3 (grain size interval 1) using bootstrap method.
- Figure 8.4: F-SSDs for dataset 3 (grain size interval 1) using logistic function.
- Figure 8.5: F-SSDs for dataset 4 (grain size interval 2) using bootstrap method.
- Figure 8.6: F-SSDs for dataset 4 (grain size interval 2) using logistic function.
- Figure 8.7: F-SSDs for dataset 5 (grain size interval 3) using bootstrap method.
- Figure 8.8: F-SSDs for dataset 5 (grain size interval 3) using logistic function.

**Figure 8.1:** F-SSDs for dataset 1 (common species) using bootstrap method. The solid line shows the median estimate (50%) percentile with lower (5%) and upper (95%) percentiles (dashed lines), which constitutes a 90% two-tailed percentile confidence interval. The apparent 5 % protection level and the adjusted 5 % community level are shown as lines in each figure (see Figure a).



(g) Zn

(h) Decalins



NA

THC [mg kg<sup>-1</sup>]

**Figure 8.2:** F-SSDs for dataset 1 (common species) using logistic function. The solid line shows the logistic curve and the blue and green lines indicate the apparent 5 % protection level and the adjusted 5 % community level.



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v



log concentration [mg/kg]

(l) PAH-2 (2-3 ring aromatic compounds)







**Figure 8.3**: F-SSDs for dataset 3 (grain size interval  $1 \rightarrow$  "mud") using bootstrap method. The solid line shows the median estimate (50%) percentile with lower (5%) and upper (95%) percentiles (dashed lines), which constitutes a 90% two-tailed percentile confidence interval. The apparent 5 % protection level and the adjusted 5 % community level are shown as lines in each figure.



(g) Zn

(h) Decalins



**Figure 8.4:** F-SSDs for dataset 3 (grain size interval  $1 \rightarrow$  "mud") using logistic function. The solid line shows the logistic curve and the blue and green lines indicate the apparent 5 % protection level and the adjusted 5 % community level.









5.00













**Figure 8.5:** F-SSDs for dataset 4 (grain size interval  $2 \rightarrow$  "mud – fine sand") using bootstrap method. The solid line shows the median estimate (50%) percentile with lower (5%) and upper (95%) percentiles (dashed lines), which constitutes a 90% two-tailed percentile confidence interval. The apparent 5 % protection level and the adjusted 5 % community level are shown as lines in each figure.













**Figure 8.6:** F-SSDs for dataset 3 (grain size interval  $1 \rightarrow$  "mud") using logistic method. The solid line shows the logistic curve and the blue and green lines indicate the apparent 5 % protection level and the adjusted 5 % community level.























**Figure 8.7**: F-SSDs for dataset 5 (grain size interval  $3 \rightarrow$  "fine sand –sand") using bootstrap method. The solid line shows the median estimate (50%) percentile with lower (5%) and upper (95%) percentiles (dashed lines), which constitutes a 90% two-tailed percentile confidence interval. The apparent 5 % protection level and the adjusted 5 % community level are shown as lines in each figure.





**Figure 8.8:** F-SSDs for dataset 5 (grain size interval  $3 \rightarrow$  "fine sand –sand") using logistic method. The solid line shows the logistic curve and the blue and green lines indicate the apparent 5 % protection level and the adjusted 5 % community level.



(e) Hg





Not enough data











#### 8.2 Bottom types

To investigate the influence of different bottom types on the toxicity threshold values (F-PNECs), all sediment samples were sorted into one of three bottom types based on: (1) particle size (grain size), (2) total organic matter (TOM) and (3) depth. The resulting three bottom types may broadly be characterised as (**Table 8.1**):

- (1) Bottom type 1: Very deep, muddy with high organic contents.
- (2) Bottom type 2: Deep, mixture of sand and mud with medium organic contents.
- (3) Bottom type 3: Shallow, sand with little organic contents.

Environmental	Bot	tom type	e 1 (N = 7)	72)	Bot	tom type	2(N = 4)	34)	Bottom type 3 (N = 930)			
parameter	from	to	Mean	SD	from	to	Mean	SD	from	to	Mean	SD
Particle size $(Md_{\Phi})$	8.14	10.79	9.28	0.59	2.09	8.18	4.96	0.97	0.22	5.03	3.06	0.82
TOM (mg/kg)	5.87	13.71	11.05	1.31	1.70	11.17	5.41	2.01	0.35	4.52	1.25	0.61
Depth (m)	250	450	323	35	177	398	301	42.2	63.0	265	116	36

Table 8.1: Summary of the environmental parameters for each bottom type.

The classification of the three bottom types were done with ordination analysis (Principle Component Analysis - PCA) using normalised data of all sediment samples collected in the period 1996 – 2001 containing information about the 3 environmental parameters. This resulted in 1472 sediment samples. However, sediment samples collected around the Vøring platform (16 samples) dominated the PCA-plot making it impossible to group the sediment samples. Thus, another PCA were run without these samples, i.e. with 1456 sediment samples (Fig. 8.9). Sorting the samples by PC-scores 1, 2 and 3 in three subgroups resulted in a total of 1436 samples, i.e. another 20 samples was excluded (the crosses that doesn't fall within the 3 coloured squares in Fig. 8.9). As seen in Table 8.1, Figure 8.9 and Figure 8.10, it is not possible to group the sediment samples into discrete bottom types without overlap in the three environmental variables. A description of the three environmental variables and their relationship are given at the end of this appendix.

An important presupposition of the method is that the sediment samples span over a wide range of concentrations. This was not always the case for sediment samples grouped into different bottom types. The most obvious consequence is that the data material gets to small to fulfil the two



**Figure 8.9:** Schematic result of PCA on normalised data for the three environmental parameters: particle size, depth and TOM.

criteria's defined in the main text regarding abundance (> 100 individuals) and distribution (> 30 samples). For example, for the hydrocarbon compounds, PAH, NPD, decalins, as well as Cr there was not enough data to fulfil criteria 1 and 2 for bottom type 1. However, a more subtle consequence is that even though the two criteria's are fulfilled, the data material may be insufficient estimate reliable PNECs. For to instance, the concentration range of Pb for bottom type 1 is only 25 - 54

mg/kg, i.e. there is no information about species abundance at low Pb concentrations. The same pass for Cu and Zn in bottom type 1 (Cu: 11 - 18 mg/kg; Zn: 52 - 123



Figure 8.10: Overview of three bottom types and (a) Particle size (b) Total Organic Matter (c) Depth.

mg/kg), and for THC (0 - 16 mg/kg), although for THC it is opposite, i.e. there is no information about species abundance at high THC concentrations. Consequently, although the abundance of some species decreases in the range investigated, one do not know what the scenarios are at lower (or higher) concentrations, and therefore have to extrapolate from scarce and plausible insufficient information. Thus, the impression that benthic invertebrates who spend much of their lifecycle in deep, muddy sediment with lot of organic contents (bottom type 1) is insensitive to Cu, Pb and Zn (high PNECs) and very sensitive towards THC (low F-PNEC) is due to insufficient data material (or more correct it is not possible to draw any conclusions since the PNECs is not estimated form sufficient data material).

In conclusion, the dataset was not adequate to derive reliable F-SSDs and F-PNECs and is therefore not given in this report.

#### 8.2.1 Bottom parameters

**Particle (grain) size:** The Udden-Wentworth scale is used in MOD to characterize the particle size in a sediment sample (Fig. 8.11). The particle size is given in  $\Phi$  units, where  $\Phi$  is <u>-log\_2d</u>, where d is the grain size in millimetres. From the distribution curves one may calculate different parameters such as Mean diameter and Median particle size (M<sub>z</sub> and Md<sub> $\Phi$ </sub>), standard deviation (sorting or So<sub> $\Phi$ </sub>), skewness (Sk<sub> $\Phi$ </sub>), and kurtosis (K<sub> $\Phi$ </sub>) which collectively characterize particle size of a sediment sample. The median particle size (Md<sub> $\Phi$ </sub>), which measures the central tendency of the grain size distribution is used in the present report as measurement of particle size.

**Total organic matter (TOM):** Organic matter plays a major role in aquatic systems. It affects biogeochemical processes, nutrient cycling, biological availability, chemical transport and interactions.

**Depth:** Both station and sample (actual depth when sampling) depths are listed in the MODdatabase. These were used interchangeably in order to get as much sediment samples as possible, but with actual sample depth as first rang (i.e. if both depths were listed the measured depth taken at the actual sampling was used). The sample and station depth are almost identical, so this have no practical consequences in this setting.

**Relationship between the three parameters:** The correlation matrix between the three environmental variables is given in Table 8.2. Although there is a large correlation between the environmental variables it was not possible to sort them into bottom types without using the aid of some kind of objective analysis.

<b>Correlation Matrix</b>			
	Particle size	Depth	ТОМ
Particle size	1.00 (perfect)	0.47 (almost large)	0.79 (very large)
Depth	-	1.00 (perfect)	0.71 (very large)
ТОМ	-	-	1.00 (perfect)

Table 8.2: Correlation matrix of the three environmental variables (Pearson product moment).

Note: Qualitative scale after: Cohen, J. (1988). Statistical power analysis for the behavioral sciences (2nd ed.). New Jersey: Lawrence Erlbaum.(Cohen 1988)



**Figure 8.11**: (a) Median particle size distribution and suggestion of classification (after Folk and Ward, 1957, adapted from Wentworth, 1922). (b) Cobble (c) Pebble (d) Granule (e) Sand (f) Pelite (mud) Pictures from the Internet: National Oceanic and Atmospheric Administrations (NOOA): <u>http://www.noaa.gov/</u>

### 8.3 LSC

This appendix is an extract of the document: "<u>Background levels of "heavy</u>" metals in the different regions on the Norwegian Continental Shelf".

Table 8.3 gives the LSC values in 6 of the 7 investigated regions on the Norwegian Continental Shelf. The regions are divided into sub-regions according to the description given below. The LSC values are the average of 3 years, except Reg-3a and Reg-4d, which is for hydrocarbon substances only estimated from one year.

Table 8.3: Summary of LSC values for regions and sub regions. Concentration in mg kg<sup>-1</sup>, dry weight (ppm).

	Reg1-a	Reg1-b	Reg2-a	Reg2-b	Reg3-b	Reg3-a	Reg4-a	Reg4-b	Reg4-c	Reg4-d	Reg6-a	Reg9
THC	6.40	5.10	9.80	6.60	9.50	35.10	6.50	5.20	7.30	26.60	8.30	-
NPD	0.03	0.02	-	-	0.02	0.77	0.05	0.06	0.02	0.20	0.15	-
PAH	0.06	0.05	-	-	0.03	0.79	0.10	0.14	0.03	0.72	0.20	-
Decalins	-	-	-	-	0.05	0.05	0.05	0.05	0.05	0.05	-	-
Ba	76.40	8.60	146.00	38.00	159.00	401.00	456.00	554.00	391.00	502.00	308.00	157.00
Cd			0.03	0.01	0.03	0.13	0.10	0.09	0.11	0.14	-	0.23
Cr	7.90	12.40	9.60	10.20	6.50	41.20	11.50	12.90	8.40	30.50	52.00	39.00
Cu	1.00	1.50	2.00	1.20	1.60	17.40	4.90	5.90	2.80	15.70	18.00	19.00
Hg			0.01	0.01	0.01	0.05	0.03	0.02	0.03	0.04	-	0.13
Pb	7.50	12.20	7.00	7.40	5.30	47.90	8.60	9.20	8.40	35.10	37.00	36.00
Zn	6.30	9.60	8.90	8.10	6.20	86.20	21.70	24.50	12.40	63.60	94.50	70.00

Data from the document "Background levels of heavy metals in the different regions on the Norwegian Continental Shelf"

#### 8.3.1 Limit for significant contamination and interpretation

To calculate LSC (Limit of Significant Contamination) a PCA analysis shall first be conducted for the year in question alone and combined for the entire set of data (from and including 1996) The results from the PCA analysis will show whether it is necessary to divide the individual region into sub-regions. Any sub-regions shall be the same for THC and Barium. LSC shall be calculated both with this year's set of data and the total set of data from and including 1996.

The following sequence shall be followed in selecting LSC:

- the entire region (all reference stations and regional stations)
- any sub-regions (based on selected reference stations and regional stations)
- alternatively each field-specific reference station (including the unaffected stations on the field)

The values based on the different calculation methods shall be compared, and the data material to be used as a basis for the calculation of LSC shall be chosen by discretion. LSC shall be stated with one decimal only.

LSC shall be based on mean values, using a unilateral t-test and a level of significance of 5%.

$$LSC > \overline{R}_{\bullet \bullet} + t_{\alpha(1),\nu} \cdot s \cdot \sqrt{1 + \frac{1}{N_r}}$$

 $\overline{R}_{\bullet\bullet}$  = the average of mean values for the reference/background stations.

 $t_{\alpha(1),\nu}$  = critical value from the t-distribution, using a unilateral t-test with a level of significance of  $\alpha(=0.05)$ 

and  $v = N_r - 1$  degrees of freedom.

 $N_r$  = Number of reference stations

as 
$$s = \sqrt{\frac{\sum_{i=1}^{N_r} (\overline{R}_{i\bullet} - \overline{R}_{\bullet\bullet})^2}{N_r - 1}}$$

The standard deviation s is calculated as

where  $\overline{R}_{i\bullet}$  = the mean values of the parallels at reference station no. *i*.

# 8.4 Abbreviations

#### THC =Total hydrocarbon (many compounds)

#### NPD (sum) - Naphtha Petroleum Derivates

Naftalen C1-Alkylnaftalener C2-Alkylnaftalener C3-Alkylnaftalener Fenantren Antracen C1-Alkylfenantrener/Antracener C2-Alkylfenantrener/Antracener C3-Alkylfenantrener/Antracener Dibenzotiofen C1-Alkyldibenzotiofener C2-Alkyldibenzotiofener C3-Alkyldibenzotiofener

#### PAH - Poly-aromatic hydrocarbons

Naftalen Acenaften Acenaftylen Fluoren Fenantren Antracen Fluoranten Pyren Benzo(a)antracen Krysen/Trifenylen Benzo(a)pyren Benzo(b,j,k)fluoranten Benzo(g,h,i)perylen Indeno(1,2,3-c,d)pyren

#### PAH1 (1-ring aromatic compound)

Naphthalene

#### PAH2 (2-3 ring aromatic compounds)

Acenaphthene Acenaphthylene Fluorene Phenanthrene Anthracene Fluoranthene

#### PAH3 (4-6 ring aromatic compounds)

Pyrene Benzo(a)anthracene Chrysene/Triphenylene Benzo(a)pyrene Benzo(b,j,k)fluoranthene Benzo(g,h,i)perylene Indeno(1,2,3-c,d)pyren Dibenzo(a,h)anthracene

#### PAHc (PAH<sub>13</sub> and PAH pooled).

#### **PAH13**:

Acenaften Fluoren Fenantren Antracen Fluoranten Pyren Benzo(a)antracen Krysen/Trifenylen Benzo(a)pyren Benzo(b,j,k)fluoranten Benzo(g,h,i)perylen Indeno(1,2,3-c,d)pyren Dibenzo(a,h)antracen

#### Decalins (sum of 4 compounds)

C5-Alkyldekaliner C6-Alkyldekaliner C7-Alkyldekaliner C8-Akyldekaliner

Ba = BariumCd = cadmiumCr = ChromiumCu = Cupper

Hg = Mercury

Pb = Lead

Zn = Zink