Grethe Kjeilen, Stig Westerlund, Sigfryd Torgrimsen, Anne Bjørnstad and Endre Aas

Assessment of environmental implications of Hutton TLP mooring in the Vats fjord

FINAL REPORT

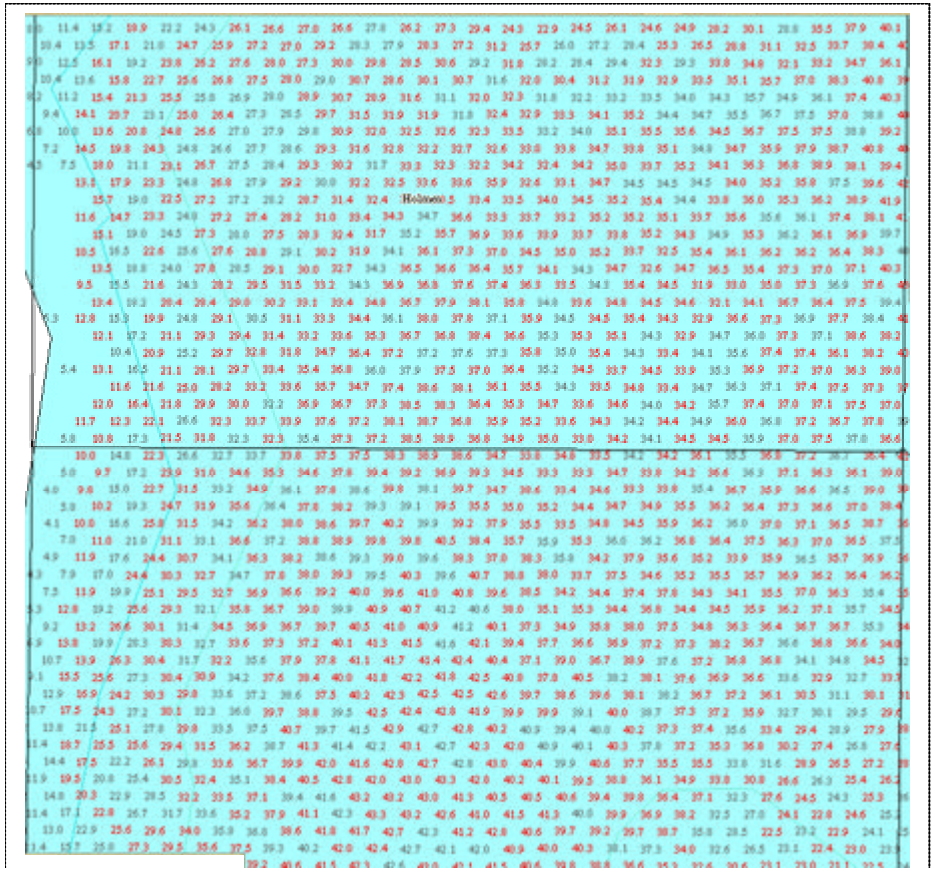
Appendix

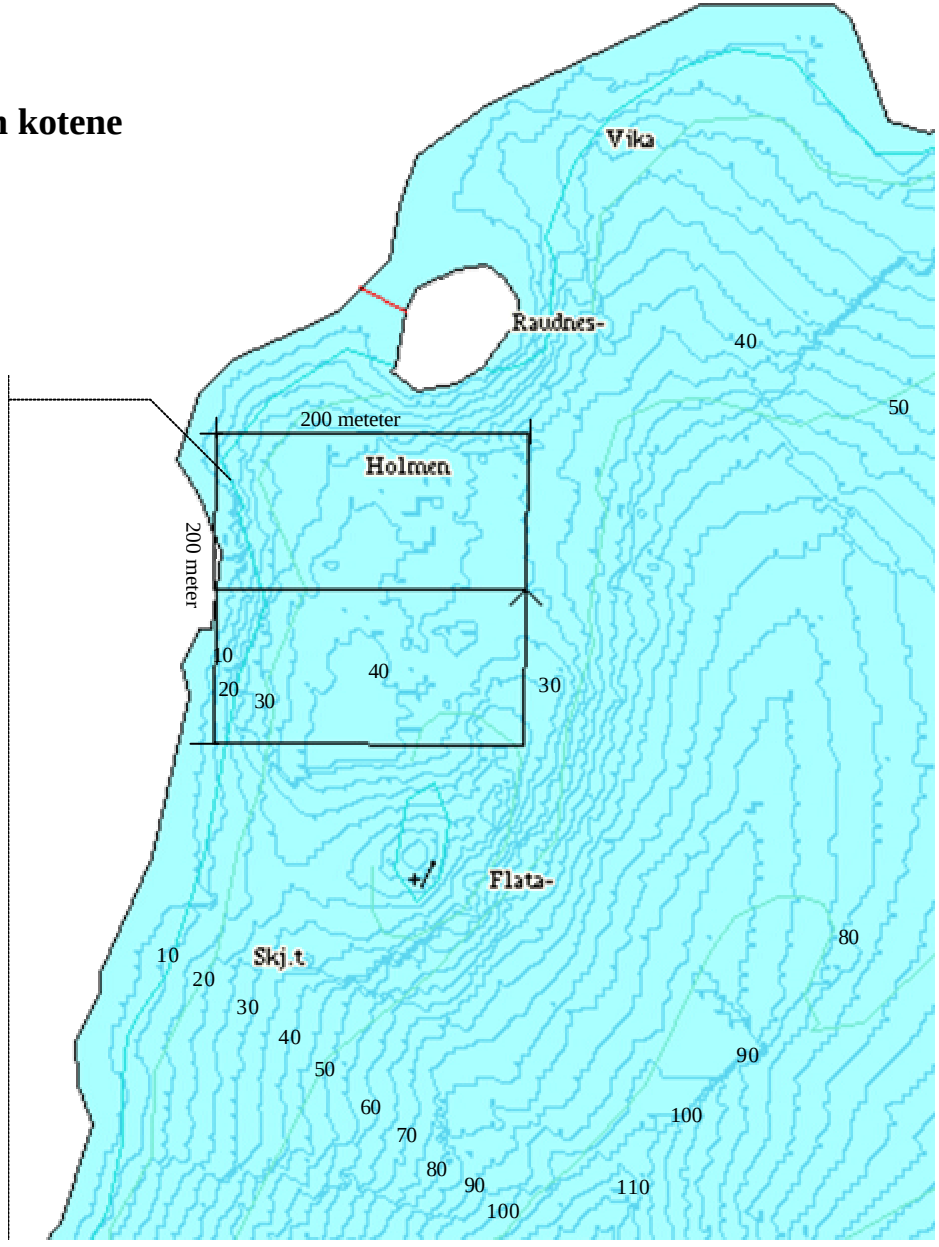
ROV-maps

Maps as reported by Amundsen Diving (following three pages, with different resolution).

N

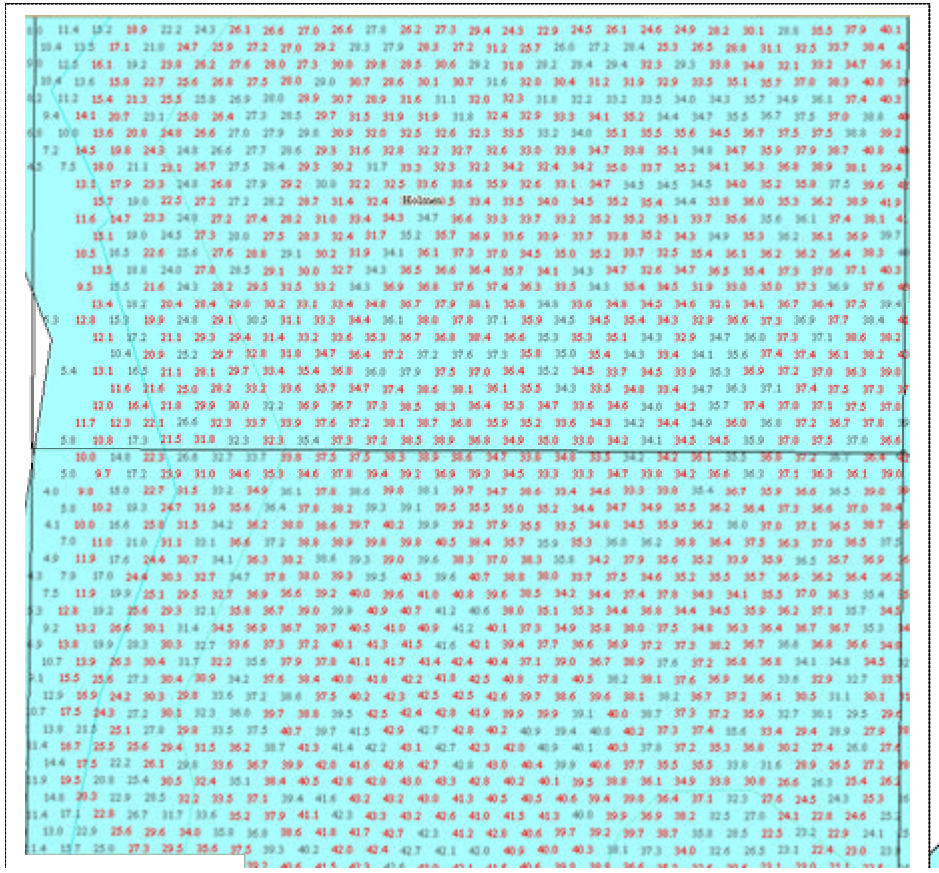
5 meter mellom kotene

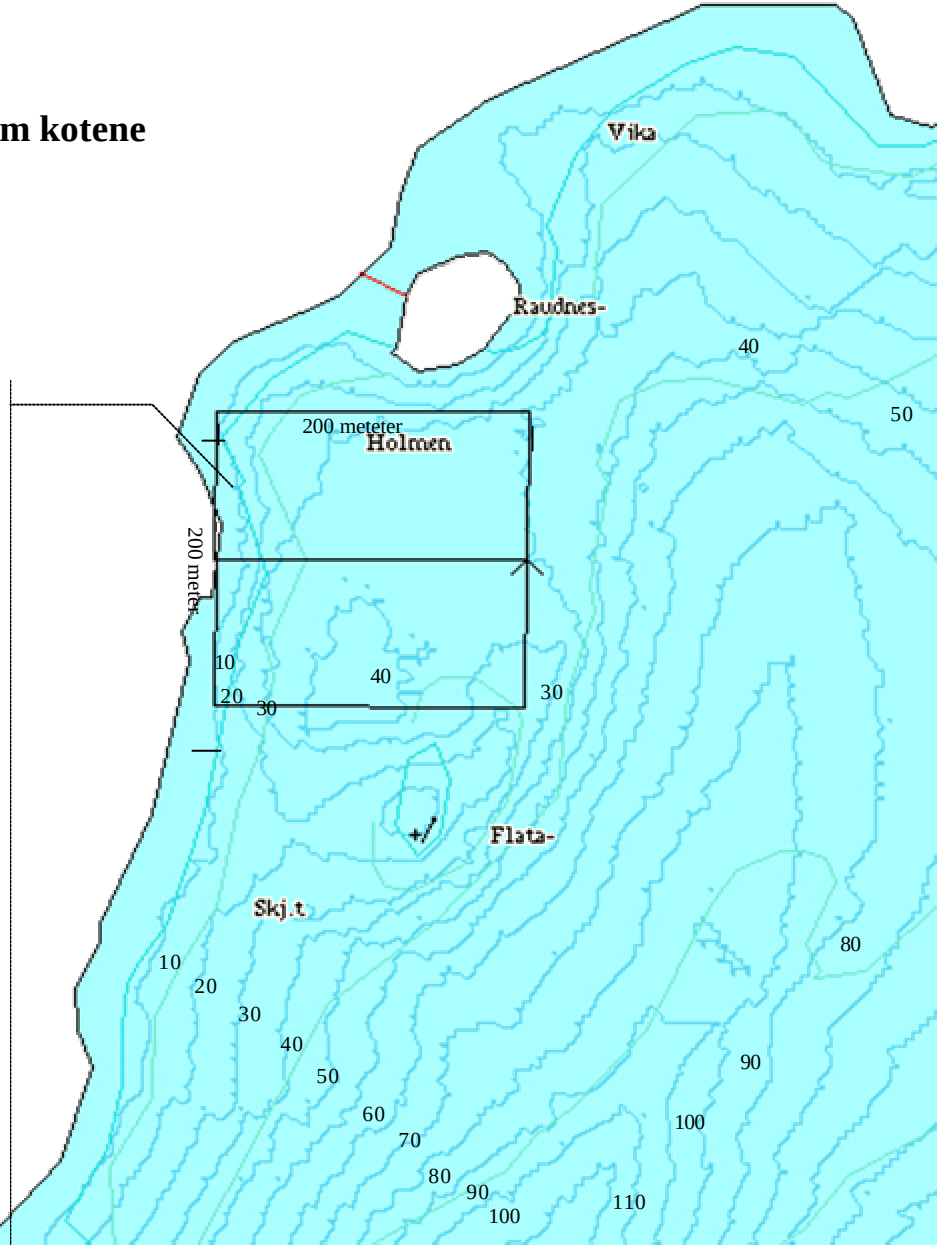


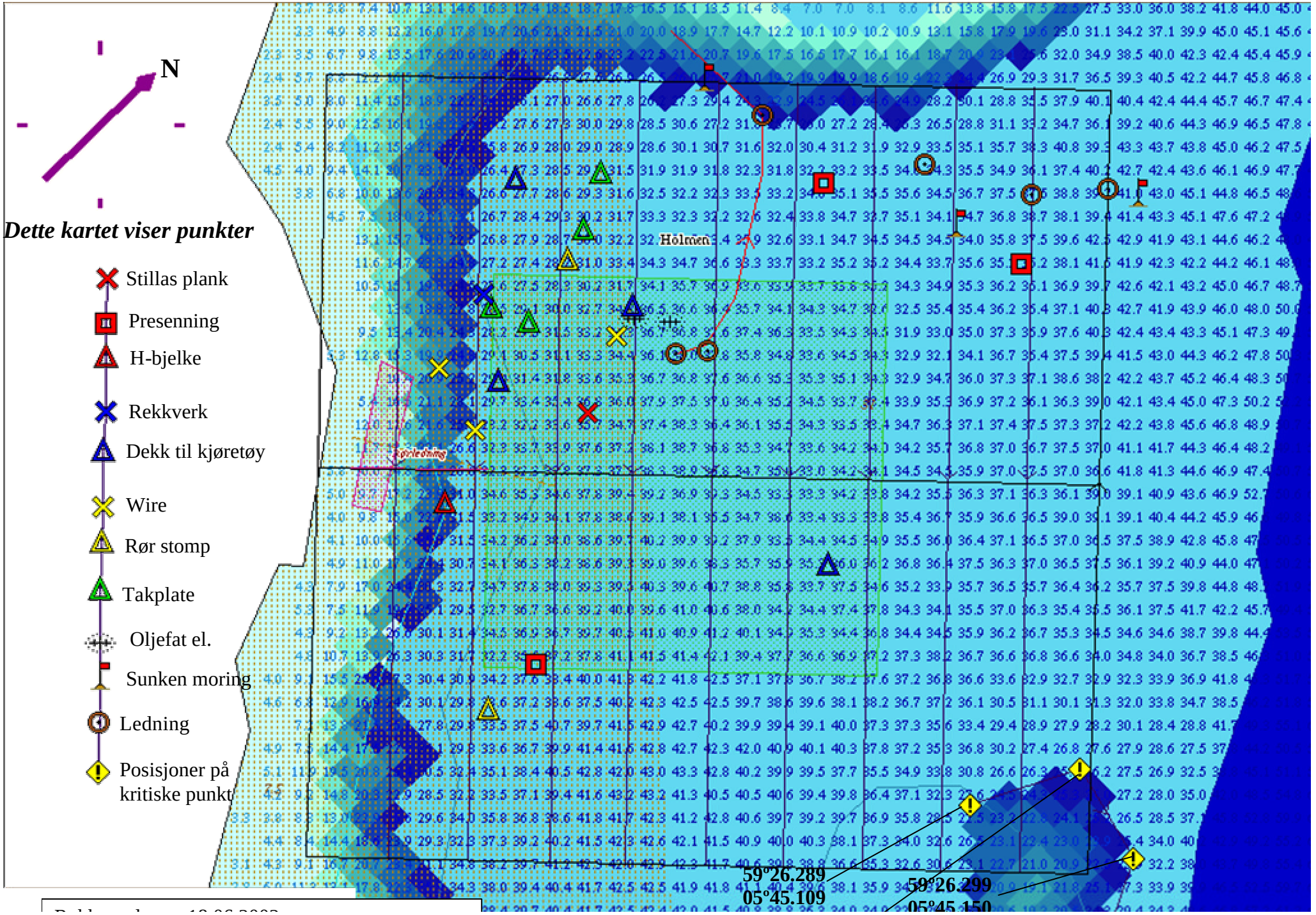


N

10 meter mellom kotene






N
Dette kartet viser punkter
Stillas plank
Presenning
H-bjelke
Rekkverk
Dekk til kjøretøy
Wire
Rør stomp
Takplate
Oljefat el.
Sunken moring
Ledning
Posisjoner på kritiske punkt
Hölmen
59°26.289
05°45.109
59°26.299
05°45.150

ORGANIC PARAMETERS

Analyserapport
Sedimentprøver

MILJØ-KJEMI
Norsk Miljø Senter

Saksnr. 350009

5 Sider

Prøvemateriale

Mottatt i lab.:	14.06.2002
Antall og prøvetype:	15/sedimenter
Prøvemerking:	**Se resultatdel**
Analysedato:	17.06.2002-01.07.2002

Metoder

THC:①	MK-2000	Analyseusikkerhet (RSD)	12 %
Tørrstoff, %	MK-4031	Analyseusikkerhet (RSD):	5 %
PAH og PCB	MK-2004	Analyseusikkerhet (RSD):	12 % for PAH og 15 % for PCB

Usikkerheten defineres som det relative totale standardavviket for konsentrasjoner større enn 10 ganger deteksjonsgrensen.

①: Analysen er ikke omfattet av akkrediteringen i sediment.

Prøveopparbeidelse

For samtlige prøver er prøve til analyse basert på tilfeldig uttak av 10 delprøver.

Det ble sortert fra 14,2 % stein fra prøven merket **S5 02206-11** før delprøve til analyse ble tatt ut. Resultatene er **ikke** korrigert for dette.

Vedlegg

GC/FID-kromatogram av prøven, blindprøve og standardblandinger er vedlagt.

Resultater

Se etterfølgende sider

Resultater

Tabell 350009-1 Enhet: **mg/kg TS**	Prøvemerking					Det. grense
	S1/02206-1	**S1/02206-2**	**S1/02206-3**	**S2/02206-4**	**S2/02206-5 ④**	
PAH:						
Naftalen	0,0040	0,017	0,0071	0,0097	0,012	0,002
Acenaftylen	0,0036	<	<	<	0,0045	0,002
Acenaften	<	<	0,0020	0,0023	0,022	0,002
Fluoren	0,0025	0,0021	0,0030	0,0029	0,025	0,002
Fenantren	0,024	0,016	0,019	0,028	0,29	0,002
Antracen	0,015	0,0071	0,0084	0,0093	0,053	0,002
Fluoranten	0,037	0,031	0,026	0,044	0,35	0,002
Pyren	0,033	0,027	0,022	0,035	0,24	0,002
Benzo(*a*)antracen	0,021	0,017	0,013	0,019	0,080	0,002
Krysen/trifenylen	0,018	0,017	0,013	0,023	0,12	0,002
Benzo(*b+j+k*)fluoranten	0,034	0,038	0,030	0,049	0,21	0,002
Benzo(*a*)pyren	0,020	0,020	0,015	0,023	0,11	0,002
Indeno(*1,2,3,cd*)pyren	0,011	0,012	0,011	0,17	0,069	0,002
Benzo(*ghi*)perylen	0,012	0,013	0,011	0,017	0,072	0,002
Dibenzo(*ah*)antracen	0,0031	0,0038	0,0032	0,0053	0,022	0,002
Sum PAH_{16} ②	**0,24**	**0,22**	**0,18**	**0,44**	**1,7**	
PCB:						
PCB no 28	<	<	<0,002 ③	<	<0,006 ③	0,001
PCB no 52	<	<	<	<	<	0,001
PCB no 101	<	<	<	<	<	0,001
PCB no 118	<	<	<	<	<	0,001
PCB no 138	<	<	<	<	<	0,001
PCB no 153	<	<	<	<	<	0,001
PCB no 180	<	<	<	<	<	0,001
Sum PCB_7 ②	**i.p.**	**i.p.**	**i.p.**	**i.p.**	**i.p.**	
THC ①	<	30	28	33	26	5-25 ③
Tørrstoff, %	78,5	73,0	72,8	68,8	61,4	0,002

① Deteksjongrensen for THC er 5 mg/kg TS i intervallet benzen - n-C_{10}, 10 mg/kg TS fra n-C_{10}-n-C_{16} og 25 mg/kg TS for n-C_{16}-n-C_{35}

② Verdier mindre enn deteksjonsgrensen inngår ikke i summen

③ Deteksjonsgrensen er forhøyet pga interferens.

④ RSD økes til 20% pga analysetekniske problemer.

TS = Tørrstoff.

<: Mindre enn den oppgitte deteksjonsgrensen.

i.p.: Ikke påvist

Tabell 350009-2 Enhet: **mg/kg TS**	Prøvemerking					Det. grense
	S2/02206-6	**S3/02206-7**	**S3/02206-8**	**S3/02206-9**	**S5/02206-11**	
PAH:						
Naftalen	0,0089	0,0077	0,24	0,0041	0,014	0,002
Acenaftylen	<	<	<	<	<	0,002
Acenaften	0,010	<	0,023	<	0,0034	0,002
Fluoren	0,011	<	0,027	<	0,0051	0,002
Fenantren	0,050	0,0088	0,16	0,0069	0,027	0,002
Antracen	0,025	0,0067	0,081	0,0066	0,012	0,002
Fluoranten	0,071	0,020	0,21	0,018	0,045	0,002
Pyren	0,058	0,019	0,17	0,015	0,038	0,002
Benzo(*a*)antracen	0,037	0,013	0,15	0,012	0,027	0,002
Krysen/trifenylen	0,033	0,013	0,13	0,011	0,027	0,002
Benzo(*b+j+k*)fluoranten	0,070	0,039	0,25	0,027	0,063	0,002
Benzo(*a*)pyren	0,035	0,015	0,15	0,012	0,031	0,002
Indeno(*1,2,3,cd*)pyren	0,024	0,17	0,075	0,011	0,023	0,002
Benzo(*ghi*)perylen	0,024	0,018	0,073	0,011	0,023	0,002
Dibenzo(*ah*)antracen	0,0068	0,0047	0,030	0,0031	0,0076	0,002
Sum PAH_{16} ②	**0,46**	**0,33**	**1,8**	**0,14**	**0,35**	
PCB:						
PCB no 28	<0,002 ③	<0,002 ③	<0,002 ③	<0,006 ③	<0,006 ③	0,001
PCB no 52	<	<	<	<0,002 ③	<	0,001
PCB no 101	<	<	<	<	<	0,001
PCB no 118	<	<	<	<	<	0,001
PCB no 138	<	<	<	<	<	0,001
PCB no 153	<	<	<	<	<	0,001
PCB no 180	<	<	<	<	<	0,001
Sum PCB_7 ②	**i.p.**	**i.p.**	**i.p.**	**i.p.**	**i.p.**	
THC ①	<	37	38	32	56	5-25 ③
Tørrstoff, %	65,3	58,7	61,1	68,4	69,4	0,002

① Deteksjongrensen for THC er 5 mg/kg TS i intervallet benzen - n-C_{10}, 10 mg/kg TS fra n-C_{10}-n-C_{16} og 25 mg/kg TS for n-C_{16}-n-C_{35}

② Verdier mindre enn deteksjonsgrensen inngår ikke i summen

③ Deteksjonsgrensen er forhøyet pga interferens.

TS = Tørrstoff.

<: Mindre enn den oppgitte deteksjonsgrensen.

i.p.: Ikke påvist

Tabell 350009-3 Enhet: **mg/kg TS**	Prøvemerking					Det. grense
	S5/02206-12	**S5/02206-13**	**S6/02206-14**	**S6/02206-15**	**S6/02206-16**	
PAH:						
Naftalen		0,013	<	0,0041	0,0042	0,002
Acenaftylen		<	<	0,0086	0,0021	0,002
Acenaften		0,0032	<	0,0021	0,0038	0,002
Fluoren		0,0044	<	0,0055	0,0056	0,002
Fenantren		0,083	0,0023	0,041	0,079	0,002
Antracen		0,022	<	0,027	0,041	0,002
Fluoranten		0,12	0,0058	0,052	0,17	0,002
Pyren		0,090	0,0061	0,050	0,15	0,002
Benzo(*a*)antracen		0,054	0,0063	0,027	0,14	0,002
Krysen/trifenylen		0,058	0,0061	0,025	0,18	0,002
Benzo(*b+j+k*)fluoranten		0,10	0,020	0,046	0,18	0,002
Benzo(*a*)pyren		0,047	0,0071	0,025	0,11	0,002
Indeno(*1,2,3,cd*)pyren		0,025	0,0059	0,013	0,048	0,002
Benzo(*ghi*)perylen		0,023	0,0061	0,015	0,054	0,002
Dibenzo(*ah*)antracen		0,0094	<	0,0040	0,026	0,002
Sum PAH_{16} ②	**ca 0,27**	**0,65**	**0,066**	**0,35**	**1,2**	
PCB:						
PCB no 28		<	<	<0,002 ③	<0,002 ③	0,001
PCB no 52		<	<	0,0015	<	0,001
PCB no 101		<	<	0,0039	<	0,001
PCB no 118		<	<	0,0044	<	0,001
PCB no 138		<	<	0,0060	<	0,001
PCB no 153		<	<	0,0049	0,0013	0,001
PCB no 180		<	<	0,0024	<	0,001
Sum PCB_7 ②	**i.p.**	**i.p.**	**i.p.**	**0,023**	**0,0013**	
THC ①	<	39	<	<	<	5-25 ③
Tørrstoff, %	64,4	69,0	76,2	74,2	72,7	0,002

① Deteksjongrensen for THC er 5 mg/kg TS i intervallet benzen - n-C_{10}, 10 mg/kg TS fra n-C_{10}-n-C_{16} og 25 mg/kg TS for n-C_{16}-n-C_{35}
② Verdier mindre enn deteksjonsgrensen inngår ikke i summen
③ Deteksjonsgrensen er forhøyet pga interferens.
TS = Tørrstoff.
<: Mindre enn den oppgitte deteksjonsgrensen.
i.p.: Ikke påvist

Oslo, Juni 2002

MILJØ-KJEMI, Norsk Miljø Senter

Eva Kristin Løvseth
cand. scient.

Einar Richter Jordfald
avdelingsleder

BIOLOGIC PARAMETERS

Analysis of PAH metabolites in crab urine

Urine was diluted 10 times in 50 % methanol and fluorescence analysed by a Perkin Elmer LS50 B Luminescence Spectrophotometer. The following wavelength pairs (excitation/emission) were used for the fixed wavelength fluorescence (FF) analyses: 290/335 nm (naphthalene/ phenanthrene type of metabolites), 341/383 nm (pyrene type of metabolites) and 380/430 nm (BaP type of metabolites). A slit width of 2.5 nm (ex/em) was applied, and samples were measured in a 0.8 ml quartz cuvette. Synchronous fluorescence spectrography (SFS) was analysed on a selection of the samples. A difference between excitation and emission wavelength of 42 nm was applied. The fluorescence signals were semi-quantitatively estimated to pyrene fluorescence equivalents (PFE) for each of the three wavelength pairs. A standard curve was made with commercially available pyrene (Sigma, St.Louis USA) measured at its optimal wavelength pairs 332/374 nm. Cuvette, slit width and solvent were identical to the sample analyses.

The Neutral Red Lysosomal Retention Assay

The ability of cells to take up and retain the cationic vital dye, neutral red, has been used in several studies as a measure of cytotoxicity (Dierickx and Van De Vijver, 1991). More recent work by Lowe *et a*l. (1992) demonstrated the use of the neutral red assay as an indicator of contaminant exposure in fish. Further work has developed the technique for use on invertebrate cells (Lowe and Pipe, 1994) as a reliable indicator of invertebrate health in laboratory and field studies.

Haemolymph extraction procedure

Haemolymph is drawn from the posterior adductor muscle (PAM) of *Mytilus edulis* by the following procedure:

a) The shell is opened using a small scalpel or scissors,

b) A syringe filled with 0.1 ml physiological saline (4.77g/l Hepes (99%), 1.47g/l $CaCl_2$, 13.06g/l $MgSO_4$, 25.48 g/l NaCl and 0.75g/l KCl, pH 7.36) is used to withdraw 0.1 ml haemolymph from the muscle (PAM). If 0.1 ml cannot be obtained, or more haemolymph is required, then the target should be to achieve a **50:50** mixture of hamolymph and saline. If repeated bleeding is required from an individual then extra care should be taken during haemolymph extraction, Smaller specimens, particularly in the case of *M. edulis*, may prove difficult to rebleed and it is suggested that adequately sized animals are chosen to suit the experimental requirements (> 4 cm in length).

c) After removal of the needle (to reduce shear stress) haemolymph samples are rapidly dispensed into an eppendorf tube after each bleeding. The haemolympg extracting procedure should be carried out in a rapid, efficient manner, the aim being to remove the haemolymph without unduly stressing the specimen and compromising the sample. It should not take an experienced operator more than 1-2 minutes to extract haemolympf from one individual.

Slide preparations

a) Slides are treated with a working 1:10 dilution of Poly-L-lysine in destilled water, 1 droplet applied to each slide, smeared with a cover slip, and then allowed drying.

b) **30-50** m**l** of cell suspension (haemolymph/saline mixture) is then transferred from the eppendorf and onto the centre of the treated microscope slides. The cell suspension is left in a light proof humidity chamber at 20^{o}C (room temperature) for **15 minutes** to allow adherence of the cells. A humid atmosphere can be obtained by placing wet paper towels in the base of the chamber. The chamber is supposed to prevent the haemolymph samples from drying out and photo-activation of the neutral red probe.

c) After 15 minutes the slides are removed individually from the chamber and the excess suspension is carefully tapped off. Holding one end of the slide, it is tapped lightly on a paper towel on the benchtop. This will leave a monolayer of cells on the slide. The slides are quickly returned to the humidity chamber.

d) **30-50**m**l** of a working solution of the probe, Neutral Red (10 µl Stock solution into 5 ml physiological saline) is added to the cell layer, and finally a 22 x 22 mm coverslip placed on the slide

Visualisation of Neutral Red Retention time

- After 15 minutes incubation in the lightproof humidity chamber, slides are examined systematically using light microscopy. The coloured cells are located using lower power (x10/20) and then examined using x40/100 magnification. The light level is kept to a minimal tolerable level, recorded and maintained throughout the subsequent analysis. Examination time for each slide should be kept to less than a minute.

- Following a further 15 min incubation, the preparation are examined again and thereafter systematically at 30 minutes intervals to determine at what point in the time there is evidence of dye loss from the lysosomes to the cytosol.

- The test for each slide terminated when dye loss is evident in 50% (numerically assessed within each field view) of the granular haemocytes, and the time is recorded when this occurs.

- The mean retention time (NRRT) is then calculated for each sample set. A healthy sample set of *M.edulis* normally has a retention time from 150-180 minutes.

NB Spawning animals should not be included in any tests, because NRRT will normally decrease dramatically when the animals are spawning. The lysosomes are enlarged and will start leaking were quickly

References

DIERICKX, P. J., VAN DE VIJVER, I. (1991). Correlation of the neutral red uptake inhibition assay of cultured fathead minnow cells with fish lethality tests. *Bull. Environ. Contam. Toxicol.* 46, 649-653.

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