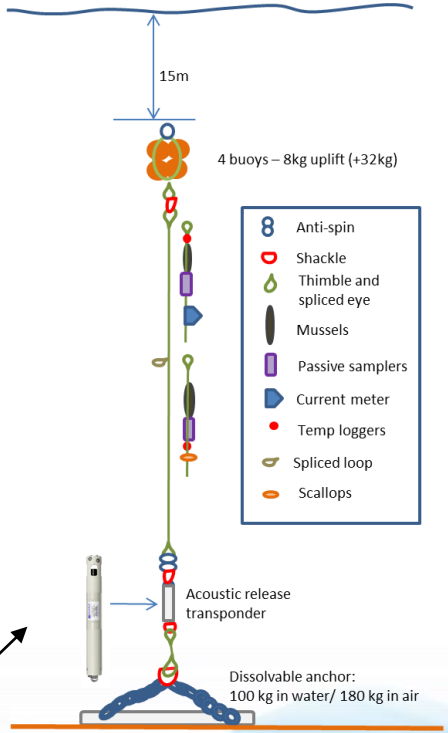
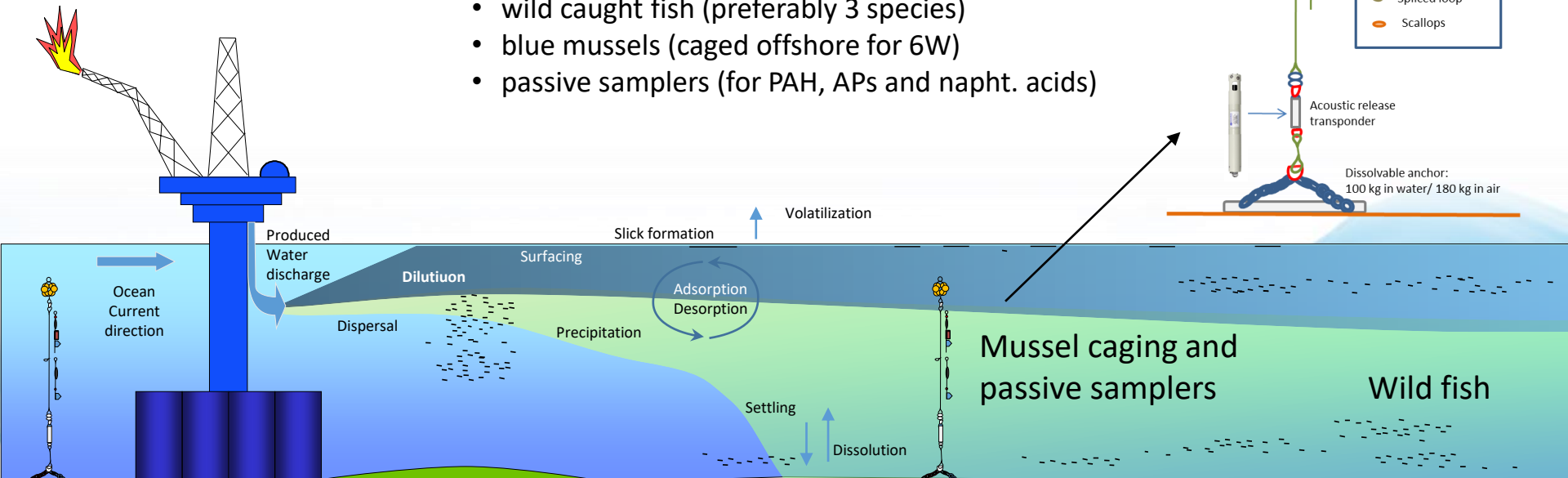
Biomarker SOPs in water column monitoring (WCM) program

Forum for Offshore Monitoring, 19-20 october 2021

Key samples collected/analyzed in WCM surveys:

- wild caught fish (preferably 3 species)
- blue mussels (caged offshore for 6W)
- passive samplers (for PAH, APs and napht. acids)





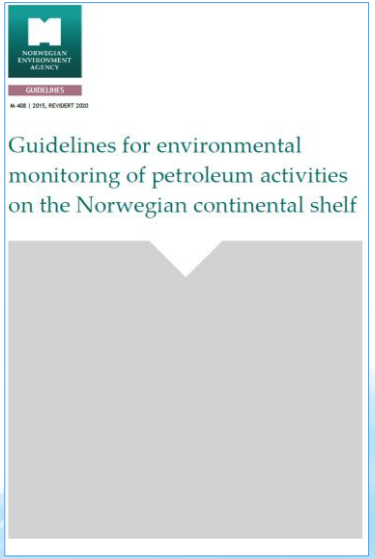
Definition Standard Operating Procedure (SOP)

- A SOP is a document that describe in detail how a procedure should be done.
- The SOP may regard both analysis and non-analysis procedures.
- There are no formal demands to the content of a SOP, but it is normally written as a step-by-step procedure recipe.
- The SOP should include all the relevant information that makes it feasible for personnel to execute the given procedure correctly.
- The SOP should include information about the modifications and improvements done on the procedure document over time.

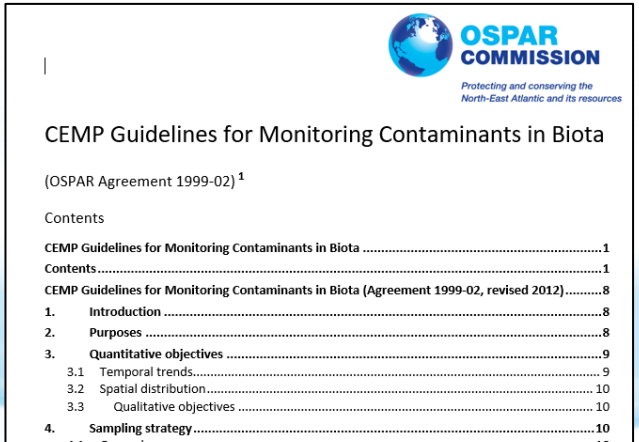
Monitoring guidelines from the Norwegian Environment Agency provide general guidance for how monitoring surveys in offshore water column and sediments should be done

- The guidelines do not themselves provide technical instructions (e.g., standard operating procedures, SOPs) for chemical and ecotoxicological parameters that are required or recommended by the guidelines.
- M-300/M-408 refer to other guidelines for providing instructions on analyses methods or for QA of WCM parameters, especially the two CEMP Guidelines OSPAR Agreement 1999-2 and OSPAR Agreement 2002-15.
- **But these CEMP guidelines don't include biomarker-relevant instructions or SOPs.**

M-300/M-408 Guidelines



Revised most recently in 2020



OSPAR COMMISSION
Protecting and conserving the North-East Atlantic and its resources

CEMP Guidelines for Monitoring Contaminants in Biota

(OSPAR Agreement 1999-02) [1]

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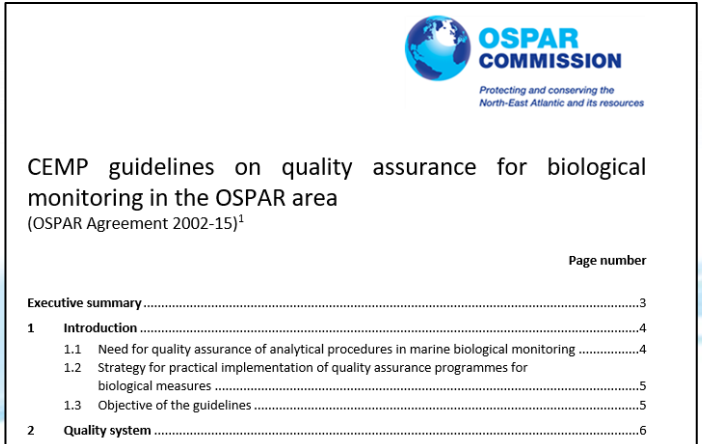

OSPAR COMMISSION
Protecting and conserving the North-East Atlantic and its resources

CEMP guidelines on quality assurance for biological monitoring in the OSPAR area
(OSPAR Agreement 2002-15)[1]

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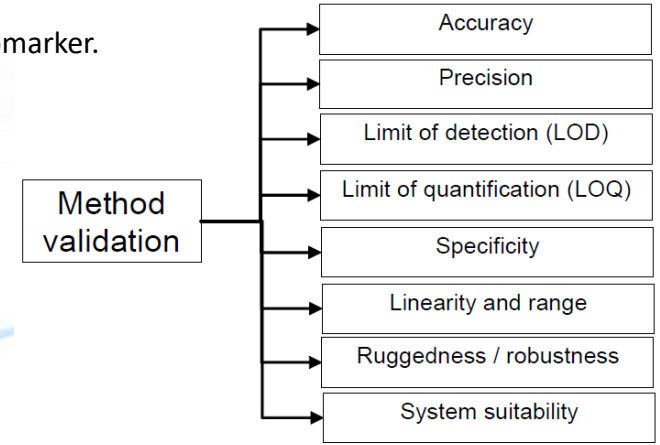
1 Introduction 4
1.1 Need for quality assurance of analytical procedures in marine biological monitoring 4
1.2 Strategy for practical implementation of quality assurance programmes for biological measures 5
1.3 Objective of the guidelines 5
2 Quality system 6

There is need for consistency of analysis method descriptions ..

- **The M-300/M-408 guidelines state that WCM reports shall include a brief description of the laboratory procedures for physical, chemical and biological analyses, including description of any deviations, with reasons, and an evaluation of whether/how results are affected.**
  - Brief description = shorter version of full method description, with reference to full description.
  - Enough technical/procedural info should be available, so others are able to perform similar analyses.
  - Method accessibility important for enabling critical assessments of reported WCM data.
  - The objective of making a SOPs collection of WCM relevant biomarkers is old, but not yet achieved.
  - Developments and better accessibility to SOPs will facilitate biomarker quality related activities, such as:
  - The *fit for purpose* for each biomarker parameter.
  - Optimization of specific methodological and analytical conditions for each biomarker.
  - Documentation and monitoring of laboratory quality.
  - A biomarker SOP collection should be managed by Miljødirektoratet.



WCM parameters mussels

Table 3-1: Parameters to be analyzed in mussels

	Parameter	Type of tissue/matrix	Method
1	Size and condition (CI)	Whole mussel	Length of the mussel Dry weight of soft tissue on representative sub-sample shall be used to calculate condition index.
2	Speciation	Representative subsample. Individual level.	Distribution of mussels at different stations shall be done so that one can expect the same species composition at all stations. Determination of species / subspecies / hybrid composition is carried out on a representative subsample.
3	Reproductive maturity & spawning status	Whole mussel, internal organs, gonad products (histology sample to be taken as transverse incision)	A histological sample must be taken of all individuals being tested. Maturity and spawning status must be validated after the survey.
4	General health status	Whole mussel	«stress on stress»
5	PAH	Soft tissue	GC-MS A reference sample must be used when measuring PAH in mussels.
6	Metals (Hg, Pb, Cd, Ba)	Soft tissue	ICP A reference sample must be used when measuring metals in mussels.
7	Micronuclei (MN) Chromosome damage	Non-granular haemocytes. Alternatively, cells extracted from enzymatically treated gill tissue.	Manual or automatic quantification of micronuclei formation. Manual MN-scoring must be performed blindly by anonymizing and randomizing samples
8	Lysosomal membrane stability (LMS)	Digestive gland	Histology of cryostat sections of the digestive gland

WCM parameters fish

Table 3-2: Parameters to be analyzed in fish

	Parameter	Type of tissue /matrix	Method
1	Size	Whole fish	Weight, length
2	Gender	Whole fish and gonads	Macroscopic assessment, possibly Lupe
3	Age	Otolith	Microscopical assessment
4	Liver somatic index (LSI)	Whole fish and liver	Liver weight/body weight
5	Gonad somatic index (GSI)	Whole fish and gonads	Gonad weight/body weight
6	Condition index (CI)	Whole fish	Weight/length
7	PAH/NPD	Liver[2]	GC-MS
8	Concentration of PAH metabolites	Bile	GC-MS/LC-FD Stomach content shall be registered (full/empty)
9	Tissue changes, including lysosomal changes	Liver	Histology
10	CYP1A1-induction	Liver S9 or microsomes	EROD-activity
			CYP1A ELISA
			qPCR: AH-receptor gene and CYP1A1-gene
11	DNA damage	Liver	DNA adducts
		Lymphocytes	DNA strand breaks (comet)
12	Chromosome damage	Red blood cells	Manual or automatic quantification of micronuclei formation. Manual MN-scoring must be performed blindly by anonymizing and randomizing samples
13	Acetylcholine esterase (AChE) inhibition	Muscle	AChE-activity

Green color = have obtained an initial SOP

SOPs are short but should include enough info to allow other analysts to perform the analysis and (preferably) to facilitate QC and QA routines of the analysis data

Norwegian Institute for Water Research (NIVA)		NIVA-method no. A6
		Page 1 of n
Standard Operating Procedure (SOP)	Confidential	Edition no. 5
		Date: 2017-08-28
EROD (7-ETHOXYRESORUFIN O-DEETHYLASE)		Approved:

EROD (7-ETHOXYRESORUFIN O-DEETHYLASE)

1 SCOPE

Measuring 7-ethoxyresorufin-*O*-deethylase in homogenised tissue.

2 AREA OF USE

3 PRINCIPLE

4 LITERATURE

Burke, Mayer. Eggens, Galgani

5 CONTACT PERSON

Inger Lise Nerland is responsible for the method, Karina Petersen is assisting, whereas Anders Ruus is responsible for quality control (QC)/quality assurance (QA) and training of personnel.

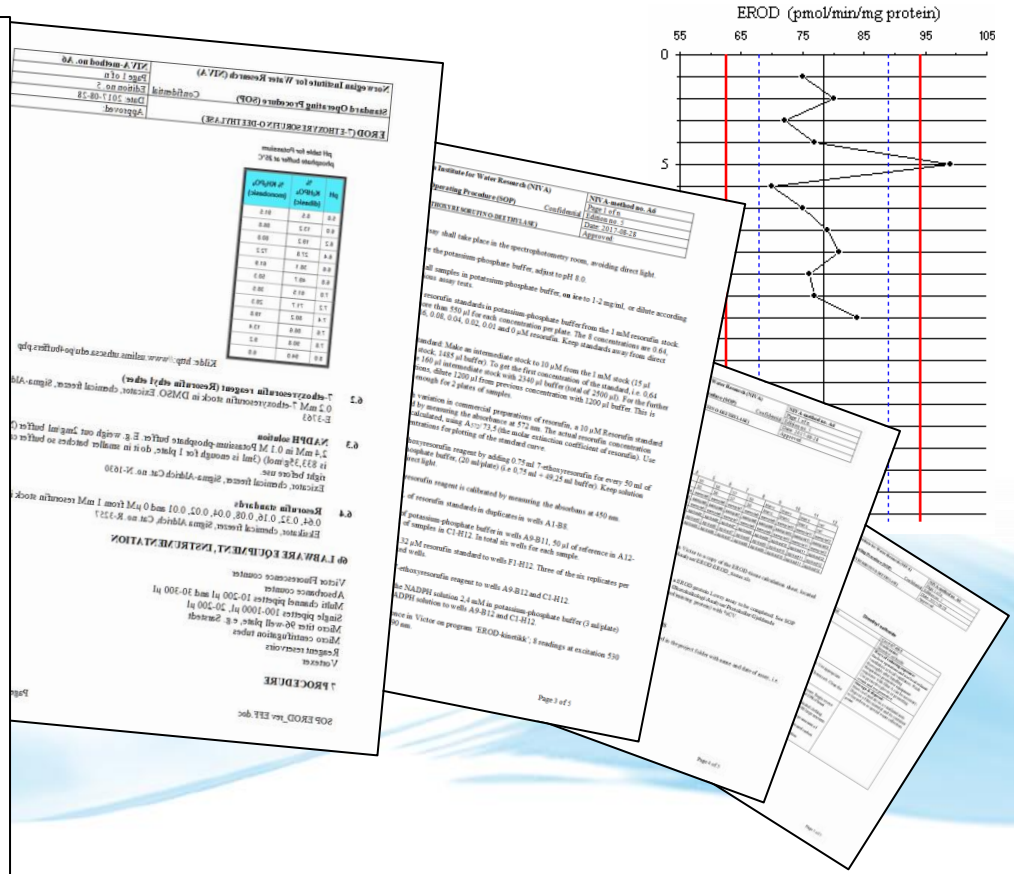
6 REAGENTS, SOLUTIONS AND STANDARDS

6.1 0,1 M Potassium-phosphate buffer pH 8.0

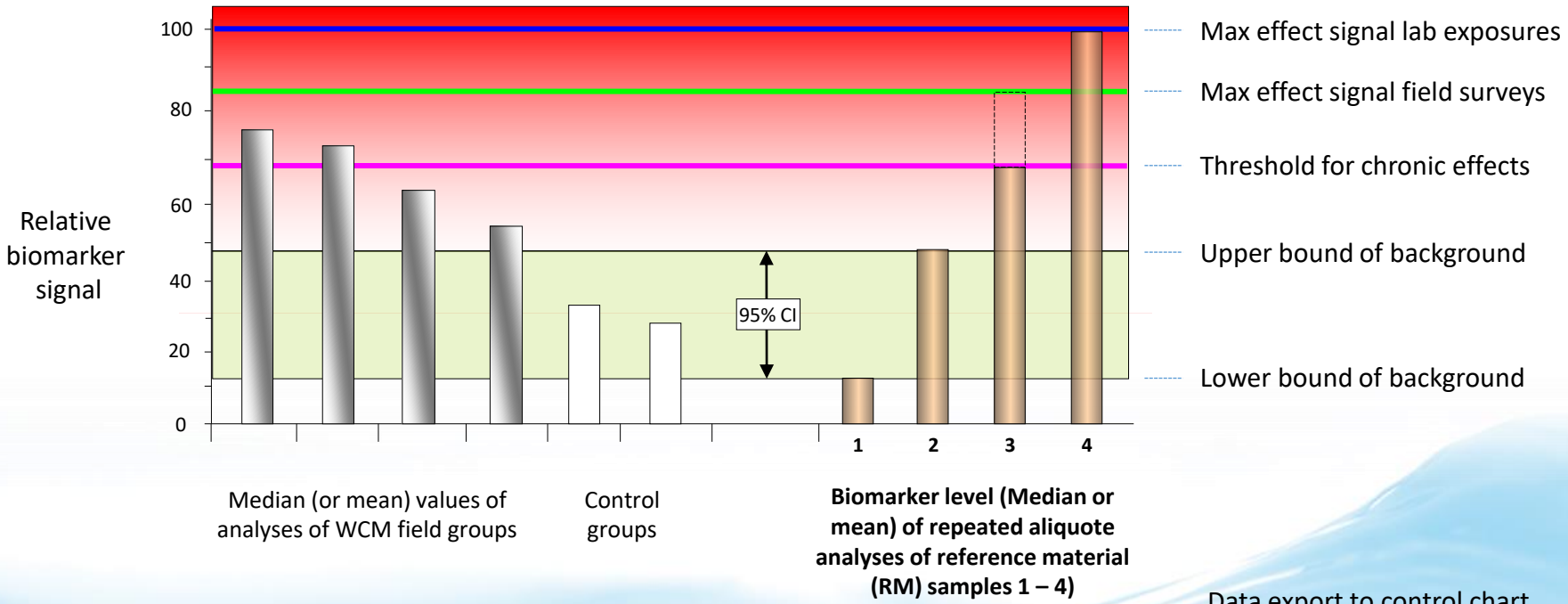
To make up 0,1 M buffer at pH 8, weigh out:

- K_2HPO_4 dibasic (174,18 g/mol) 16,373 g
- KH_2PO_4 monobasic (136,09 g/mol) 0,817 g
- Dilute to 1 L total, adjust pH if necessary

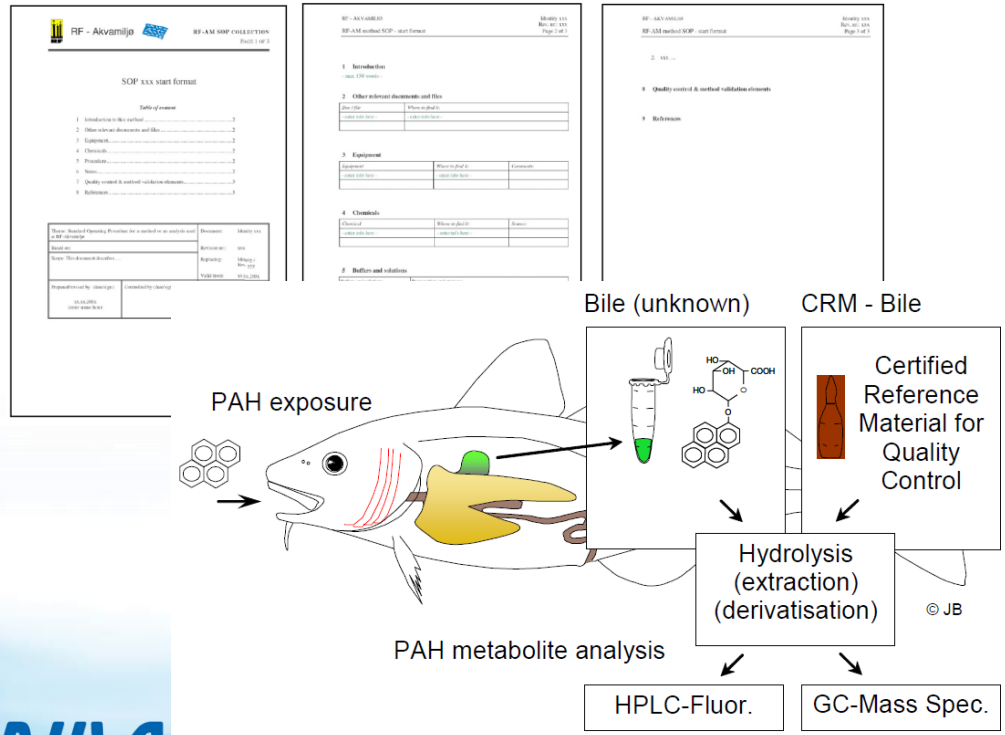
May be stored for three months. Weigh room. See table below for reference.

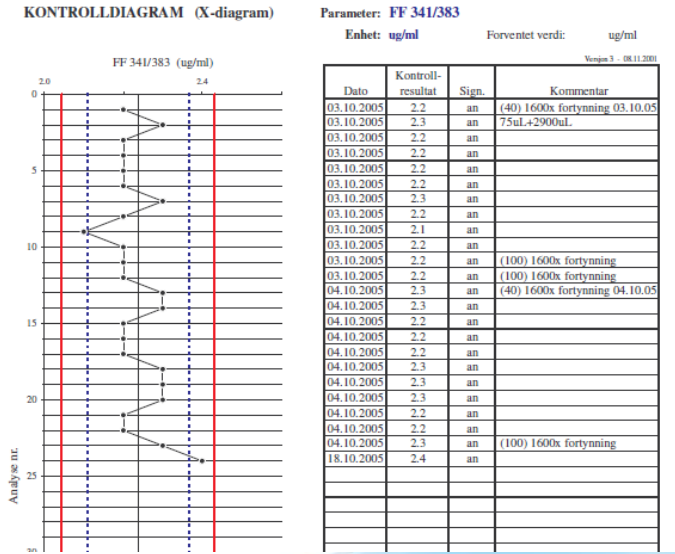


The access to SOPs simplifies preparation/use of analysis reference materials for interpretation of biomarker signals and for improved QC and QA of WCM analyses



A combined access to biomarker SOPs and RMs facilitates systematic method validation and method improvement activities

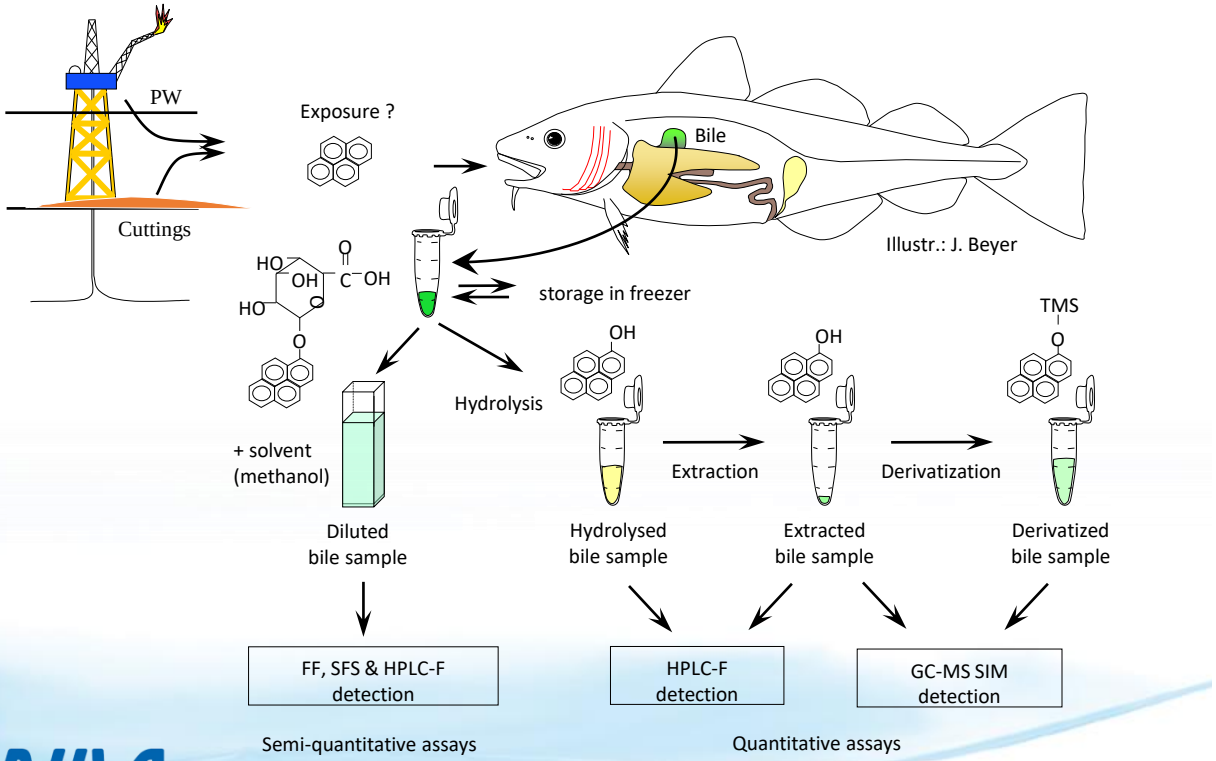


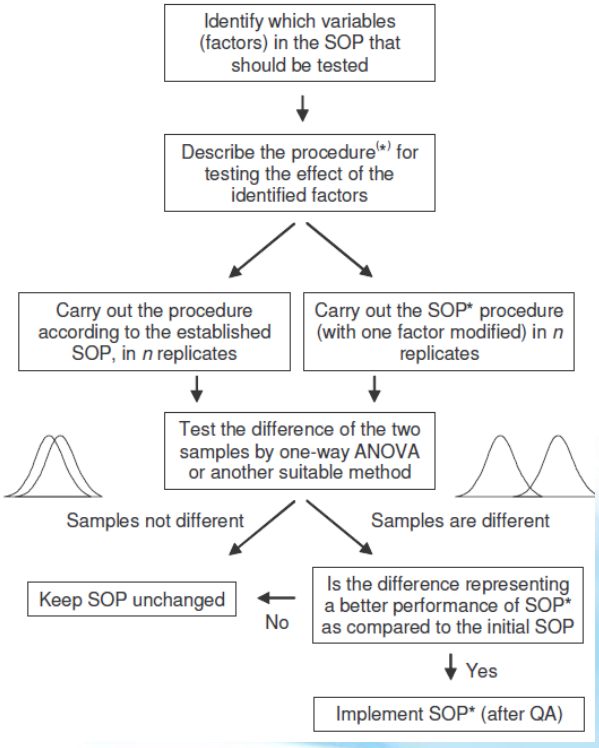


Parameter: FF 341/383
Enhet: ug/ml
Forventet verdi: ug/ml
Versjon 3 - 08.11.2001

Dato	Kontroll-resultat	Sign.	Kommentar
03.10.2005	2.2	an	(40) 1600x fortynning 03.10.05
03.10.2005	2.3	an	75uL+2900uL
03.10.2005	2.2	an	
03.10.2005	2.2	an	
03.10.2005	2.2	an	
03.10.2005	2.2	an	
03.10.2005	2.3	an	
03.10.2005	2.2	an	
03.10.2005	2.1	an	
03.10.2005	2.2	an	
03.10.2005	2.2	an	(100) 1600x fortynning
03.10.2005	2.2	an	(100) 1600x fortynning
04.10.2005	2.3	an	(40) 1600x fortynning 04.10.05
04.10.2005	2.3	an	
04.10.2005	2.2	an	
04.10.2005	2.2	an	
04.10.2005	2.2	an	
04.10.2005	2.3	an	
04.10.2005	2.3	an	
04.10.2005	2.3	an	
04.10.2005	2.2	an	
04.10.2005	2.2	an	
04.10.2005	2.3	an	(100) 1600x fortynning
18.10.2005	2.4	an	

A combined access to biomarker SOPs and RMs facilitates systematic method validation and method improvement activities





Summary - Biomarker SOPs in WCM

- Collection and systematization of SOPs for WCM is ongoing.
- Needed to clarify fit for purpose quality of mandatory biomarkers.
- Easier to standardize/optimize analytical procedures for biomarkers.
- SOPs should be free and easy-accessible (e.g., online).
- Availability of SOPs can make it easier to develop and manage biomarker analytical reference materials (RMs).
- Biomarker RMs can improve method QC, QA and effect-interpretations for priority biomarkers.
- Progress of SOP and RM matters may significantly reduce the uncertainties.

Thanks for your attention!

Method information that could strengthen the information value of WCM surveys and the WCM program in general

1. Produce fit for purpose evidence for biomarkers (and their assays) in relation to the chemical mixtures that commonly are present in discharges from offshore installations, also at field-realistic exposure concentrations.

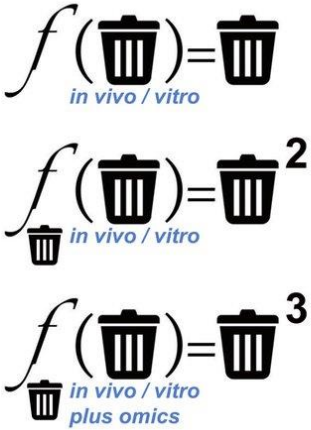
2. .. starting with the parameters listed in the M-300/M-408 guidelines.

3. Parameters/procedures that within a set deadline are not demonstrated to meet the suitability criteria must be taken out.

4. All biomarkers that meet the suitability criteria and that remain included in guidelines must be equipped with a fully quality assured analytical procedure document published in a suitable technical method website (e.g., ICES-TIMES).

5. A reference-sample program for WCM biomarkers should be established with a lifetime of 10-20 years to enable better interpretation of response intensity and better comparisons of data across years and regions.

Over-simplification, over-integration and/or other uncritical exploitations of biomarker data of possible poor quality may not be the best way forward for the WCM program



- A set of biomarkers in caged blue mussels measured according to ICES recommendations have for years been part of the offshore WCM targeting possible ecotoxicants in produced water streams discharged from offshore oil and gas production platforms. A key challenge with the use of a multi-biomarker approach is the often complex response data that are difficult to integrate in environmental policy frameworks. To encompass this problem, several ways for simplifying complex data have been developed, among which the so-called Integrated Biomarker Response (IBR) index.

Overall finds of offshore WCM surveys

- Organisms kept caged 500 - 1000 m downstream platform PW discharges develop have developed elevated markers of chemical bioaccumulation and some low-level health effects/ biomarker responses.

- These nearfield exposure effects are consistent with the discharge and risk predictions conducted by use of DREAM-EIF risk modelling.

- But, in some instances, possible PW induced alterations (such as lysosomal destabilization in saithe hepatocytes, increased biliary alkylphenol metabolites) have been detected in fish caged up to 10 km away from the PW point source.

- Increased DNA adduct levels in haddock populations in several regions of the NCS are found repeatedly, but it remains uncertain whether these putative effects are caused by contaminants originating from discharges of offshore PW, or from old oil drilling waste discharges, or from other sources.

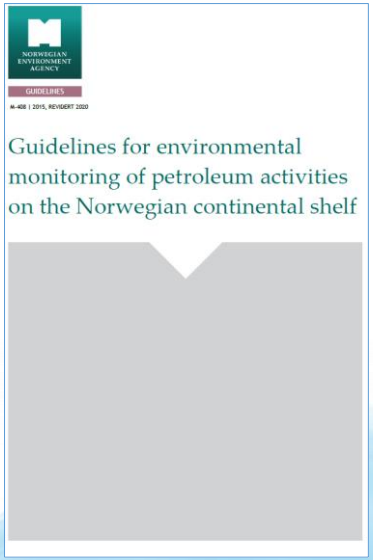
- **For WCM, there is a shortage of: evidence that show the fit for purpose for the different priority biomarkers addressed, standardized/harmonized analytical procedures for them, and suitable reference materials that can assist method QA and effect-interpretations for biomarkers that are used.**

- **The WCM results are therefore still associated with considerable uncertainty.**

Monitoring guidelines from the Norwegian Environment Agency provide general guidance for how monitoring surveys in offshore water column and sediments should be done

- The guidelines contain detailed requirements on how to carry out and report from the monitoring activities. They cover the expected scope of monitoring activities, which parameters must be analyzed, and which methods must be used, as well as provide requirements on necessary accreditation and templates for reporting.
- The QA-system should include a verification of sampling, a plan for using reference samples, reviewing analytical methods and results and performing the quality control of the report. A standard QA-system must be used, for example ISO 9000 or CEMP 2002-15.
- Quality assurance of the various analyses, both in terms of type and frequency, should be presented as part of the method description in the report.
- There is a minimum requirement that analyses are verified against reference samples run in the same test series as the real samples. The results from the reference samples must be discussed in the report from the monitoring surveys.
- All suppliers of services for monitoring programs (analyses, fieldwork) must have EN ISO/IEC 17025 accreditation, or an equivalent for the methods they use, whenever an accreditation scheme is available. Service suppliers must also document their own quality assurance and control routines. The latest and valid version of the method standards and guidelines must be used, and reference must be made to the year when these standards were established when reporting from monitoring surveys.

M-300/M-408 Guidelines



Revised most recently in 2020