REPORT SNO 6112-2011

Available NIVA generated ecotoxdata with relevance to petroleum related chemical compounds

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REPORT

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Title	Report No..	Date
Available NIVA generated ecotoxdata with relevance to petroleum related chemical compounds	6112-2011	31.01.2011
	Project No. 11061	Pages 22 Price
Author(s) Eivind Farmen	Topic group Ecotoxicology	Distribution Open
	Geographical area Norway	Printed NIVA

Client(s)	Client ref.
Akvaplan-NIVA, JoLynn Carroll	

Abstract

This report represents an overview of ecotoxicological data of petroleum-related chemical compounds generated in various NIVA projects. Specific emphasis was put on the sub-Arctic and Arctic marine environment, for applicability to the planned SYMBIOSES model system.

4 keywords, Norwegian	4 keywords, English
1. Giftighet	1. Toxicity
2. Olje	2. Oil
3. Produsertvann	3. Produced water
4. Arktis	4. Arctic

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ISBN 978-82-577-5847-9

Symbioses report

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Preface

The work described in this report has been performed on request from Akvaplan-niva, and represents an overview of ecotoxicological data of petroleum-related chemical compounds generated from various NIVA projects. Specific emphasis was put on the sub-Arctic and Arctic marine environment, for applicability to the planned SYMBIOSES model system.

Oslo, 28 January 2011

Eivind Farmen

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Summary

This report represents an overview of ecotoxicological data of petroleum-related chemical compounds generated in various NIVA projects. Specific emphasis was put on the sub-Arctic and Arctic marine environment, for applicability to the planned SYMBIOSES model system.

Sammendrag

Denne rapporten representerer en oversikt over økotoksikologiske data for petroleum-relaterte kjemiske forbindelser, generert i ulike NIVA prosjekter. Spesiell vekt ble lagt på sub-arktiske og arktiske marine miljø, for innkorporering i det planlagte SYMBIOSES modellsystemet.

1. Introduction

This project memo describes an overview of the relevant ecotoxicology data related to petroleum-related chemical compounds generated through projects conducted with NIVA as a partner during the last decade. The memo is as up-dated as possible within the time frame and resources allocated for the work organized by Akvaplan-NIVA within the SYMBIOSES project, and the overview will also be presented at the SYMBIOSES Workshop on Ecotoxicology Meta-data Analysis Exercise on 28 February – 1 March 2011.

The data generated through the various NIVA projects are based on standard experimental systems such as the marine algae Skeletonema costatum and the crustacean *Daphnia magna*, but also systems such as fish primary cell culture (*Oncorhynchus mykiss*), in vivo fish experiments with zebrafish *Danio renio*, Atlantic cod *Gadhus morhua*, as well as benthic invertebrate community structure. Skeletonema costatum are used as the test organism as they are easily cultured within the laboratory and are recommended for use in marine ecotoxicity assessments (ISO 10253, 2006. Water quality – Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*). NIVA has worked with S. costatum in toxicity studies since 1992.
Daphnia magna is the one of the most widely used organism for toxicity tests and several guidelines for both acute and chronic testing exists (OECD 202, OECD 211, ISO 6341). It is easy to keep in culture, and it reproduces by parthenogenesis making it ideal as a test organism. NIVA has worked with *D. magna* in toxicity studies since 1992.

Compounds tested vary from dispersants and drilling muds to organic extracts of produced water and synthetic produced water, reflecting the water soluble components of oil.

2. Ecotoxicity testing

2.1 Acute toxicity tests

2.1.1 Acute toxicity test with the marine algae *Skeletonema costatum*

Skeletonema costatum are used as the test organism as they are easily cultured within the laboratory and are recommended for use in marine ecotoxicity assessments (ISO 10253, 2006. Water quality – Marine algal growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum). NIVA has worked with S. costatum in toxicity studies since 1992.
A range of tests on dispersants are summarized in NIVA report 3128. Data for comparable tests with Chlamydomonas reinhardtii, where the dispersants were tested together with oil, are also present, but not summarized here due to the higher sensitivity and relevance of the marine algae Skeletonema costatum. In addition tests of drilling mud from well 7816/12-1 Rendalspasset, Svalbard was also extracted from a different report

Dispersant Arrow Emulsol LW

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	5-100 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC
Target effects	Growth rate
Type of data available	EC10: 8.4 mg/l, EC50: 29 mg/l
Data format	NIVA Report 3128

Dispersant Corexit 9527

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	3-30 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC
Target effects	Growth rate
Type of data available	EC10: 3.2 mg/l, EC50: 13 mg/l
Data format	NIVA Report 3128

Dispergant Dasic Slickgone LTS

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	3-30 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC
Target effects	Growth rate
Type of data available	EC10: 11 mg/l, EC50: 15 mg/l
Data format	NIVA Report 3128

Dispergant DisPollene 36S

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	3-30 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ±

	1 ºC
Target effects	Growth rate
Type of data available	EC10: 11 mg/l, EC50: 20 mg/l
Data format	NIVA Report 3128

Dispergant Enersperse 1037

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	1.5-55 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC
Target effects	Growth rate
Type of data available	EC10: 11 mg/l, EC50: 13 mg/l
Data format	NIVA Report 3128

Dispergant Enersperse 1583

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	3-20 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC
Target effects	Growth rate
Type of data available	EC10: 5.8 mg/l, EC50: 8.2 mg/l
Data format	NIVA Report 3128

Dispergant Finasol OSR-5

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	1.5-55 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC
Target effects	Growth rate
Type of data available	EC10: 10 mg/l, EC50: 13 mg/l
Data format	NIVA Report 3128

Dispergant Finasol OSR-12

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	3-100 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC
Target effects	Growth rate
Type of data available	EC10: 10 mg/l, EC50: 24 mg/l
Data format	NIVA Report 3128

Dispergant IKU-9

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	3-55 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC

Target effects	Growth rate
Type of data available	EC10: 18 mg/l, EC50: 25 mg/l
Data format	NIVA Report 3128

Dispergant Quell Oil C1

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	15-600 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC
Target effects	Growth rate
Type of data available	EC10: 82 mg/l, EC50: 180 mg/l
Data format	NIVA Report 3128

Dispergant Shell Dispersant VDC

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	3-55 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC
Target effects	Growth rate
Type of data available	EC10: 16 mg/l, EC50: 23 mg/l
Data format	NIVA Report 3128

Dispergant BP1100 WD

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	3-55 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC
Target effects	Growth rate
Type of data available	EC10: 23 mg/l, EC50: 49 mg/l
Data format	NIVA Report 3128

Dispergant Ameroid OSD/LT

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	15-1000 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC
Target effects	Growth rate
Type of data available	EC10: 140 mg/l, EC50: 470 mg/l
Data format	NIVA Report 3128

Dispergant Biosolve

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	1.5-20 mg/l
Experiment description	Marine algal inhibition test ISO DP 10253, 20 ± 1 ºC
Target effects	Growth rate

Type of data available	EC10: 2.4 mg/l, EC50: 3.7 mg/l
Data format	NIVA Report 3128

Drilling mud from well 7816/12-1 Rendalspasset, Svalbard was also tested in a different report:

Species and life stage	*Skeletonema costatum*
Exposure time	3 days (72 h)
Exposure concentration	3.2, 5.6, 10,18, 32, 56, 100 g/l
Experiment description	Marine algal inhibition test ISO DP 10253
Target effects	Growth rate
Type of data available	NOEC: 10 g/l, EC10: 13 g/l, EC50: 49 g/l
Data format	NIVA Report 2593

2.1.2 Acute toxicity test with *Daphnia magna*

Daphnia magna is the one of the most widely used organism for toxicity tests and several guidelines for both acute and chronic testing exists (OECD 202, OECD 211, ISO 6341). It is easy to keep in culture, and it reproduces by parthenogenesis making it ideal as a test organism. NIVA has worked with D. magna in toxicity studies since 1992.

Drilling mud from well 7816/12-1 Rendalspasset, Svalbard

Species and life stage	*Daphnia magna*
Exposure time	2 days (48 h)
Exposure concentration	3.2, 5.6, 10,18 g/l
Experiment description	Daphnia mobility inhibition test ISO6341
Target effects	Immobility/mortality
Type of data available	LC 20: 14 g/l, LC50: 23 g/l
Data format	NIVA Report 2593

2.1.3 Acute toxicity test with fish

Drilling mud from well 7816/12-1 Rendalspasset, Svalbard

Species and life stage	*Salmo trutta*, 0+, average 3.3 g
Exposure time	4 days (96 h) semi static, renewal every 24 h
Exposure concentration	10 g/l
Experiment description	OECD guidelines for testing of chemicals, No 201: Fish acute toxicity test, 10 ± 1 ºC, pH=6.3, conductivity: 3.2 ms, hardness: 11 mg CaCO3/l
Target effects	Mortality
Type of data available	No mortality observed
Data format	NIVA Report 2593

2.2 Chronic toxicity tests

2.2.1 Sediment macroinvertebrate communities

Olefin (Ultidrill, Novaplus) and ester (Anco Green, Pterofree) based drilling muds:

Species and life stage	Sediment communities
Exposure time	6 months

Exposure concentration	Approx 500 g slurry, containing 1-1.5 g drilling fluid. Approx 1.5 mm layer
Experiment description	Drill cuttings from drilling sites in the North Sea. Undistubed communities transferred from Oslofjord, 200 m depth. 7-11 ºC, salinity: 32-35 PSU
Target effects	Sediment community effect
Type of data available	Macrofauna community structure
Data format	NIVA rapport 3475-96

Olefin and ester based drilling muds:

Species and life stage	Sediment communities
Exposure time	3 months
Exposure concentration	0.5, 5, 20 mg OP cm^{-2}
Experiment description	Undistubed communities transferred from Roddenessjøen, Porsangen (Finnmark) 120 m detpth, -1.5 ºC and Oslofjord, 212 m depth. 7ºC
Target effects	Sediment community effect
Type of data available	Macrofauna number of species and individuals, diversity
Data format	NIVA rapport 3460-97

Water based drill cuttings:

Species and life stage	Sediment communities
Exposure time	6 months
Exposure concentration	3-24 mm sediment
Experiment description	Undistubed communities transferred from Oslofjord, 41 m depth. 10ºC
Target effects	Sediment community effect
Type of data available	Macrofauna number of species and individuals, diversity
Data format	Schaanning et al., 2009 J Exp Mar Biol Ecol

2.2.2 Sediment macroinvertebrate *Hediste diversicolor*

Olefin (Ultidrill, Novaplus) and ester (Anco Green, Pterofree) based drilling muds:

Species and life stage	*Hediste diversicolor*
Exposure time	6 months
Exposure concentration	Approx 500 g slurry, containing 1-1.5 g drilling fluid. Approx 1.5 mm layer
Experiment description	Drill cuttings from drilling sites in the North Sea. Undistubed communities transferred from Oslofjord, 200 m depth. 7-11 ºC, salinity: 32-35 PSU
Target effects	Biomarkers
Type of data available	Catalase activity, glutathione reductase activity
Data format	NIVA rapport 3475-96

2.2.3 Chronic mussel lab exposure: *Mytilus edulis*

Produced water from Ormen Lange gas processing plant.

Species and life stage	*Mytilus edulis*, farmed (from snadderogsnaskum)
Exposure time	5 weeks
Exposure concentration	0.01 %, 0.1 %, 0.5%, 1% PW
Experiment description	Laboratory exposure of mussels exposed to produced water from Ormen Lange gas processing plant.
Target effects	Biomarkers, chemical burdens
Type of data available	Lysosomal stability, micronuclei formation, histochemistry, peroxisomal proliferation, mussel chemistry
Data format	NIVA rapport 5747-2009

2.2.4 Chronic fish lab exposure: *Danio renio*

Water soluble components of oil, a mixture of low-molecular-weight PAHs (<5 ring) and short-chain APs (C1–C4)

Species and life stage	*Danio renio*
Exposure time	1, 7 and 13 weeks
Exposure concentration	sum PAH: 0.54 ppb (low dose), 5.4 ppb (high dose and pulsed dose).
Experiment description	Flow through exposure lasting 1, 7 and 13 weeks, 26 ± 2 ºC
Target effects	Biomarkers
Type of data available	Cytochrome P450 1A, Vitellogenin, condition, reproduction, recruitment, F1 deformations, global gene expression
Data format	Holth et al., 2008 Aq Tox

2.2.5 Chronic fish lab exposure: *Gadhus morhua*

Water soluble components of oil, low molecular weight PAHs , short chained APs

Species and life stage	*Gadus morhua*
Exposure time	4, 16, 32, or 44 weeks
Exposure concentration	Low: 0.54 ppb PAH, 1.14 ppb APs, pulsed and high: 5.4 ppb PAH, 11.4 ppb APs
Experiment description	Flow through exposure lasting 4, 16, 32, or 44 weeks
Target effects	Hepatic DNA adduct formation, Hepatic EROD activity
Type of data available	Increased DNA adduct formation following > 16 weeks exposure
Data format	Holth et al., 2009 Environ. Sci. Technol.

Water soluble components of oil, low molecular weight PAHs , short chained APs

Species and life stage	*Gadus morhua*
Exposure time	4, 16, 32, or 44 weeks
Exposure concentration	Low: 0.54 ppb PAH, 1.14 ppb APs, pulsed and high: 5.4 ppb PAH, 11.4 ppb APs
Experiment description	Flow through exposure lasting 4, 16, 32, or 44 weeks
Target effects	Biomarkers

Type of data available	Condition, gonad maturation, gene expression
Data format	Holth et al., 2010 Can J Fish Aquat Sci

2.2.6 Chronic mussel field exposure: *Mytilus edulis*

Produced water from Ormen Lange gas processing plant.

Species and life stage	*Mytilus edulis*, farmed (from snadderogsnaskum)
Exposure time	6 weeks
Exposure concentration	2 reference station, 4 Exposed stations
Experiment description	Caged mussels placed near produced water discharge point of Ormen Lange gas processing plant. Two reference sites compared to four exposed sites.
Target effects	Biomarkers, chemical burdens
Type of data available	Lysosomal stability, micronuclei formation, histochemistry, peroxisomal proliferation, mussel chemistry
Data format	NIVA rapport 5747-2009

Produced water from Ormen Lange gas processing plant.

Species and life stage	*Mytilus edulis*
Exposure time	Native mussels (not applicable)
Exposure concentration	Reference station, Exposed station 1, Exposed station 2
Experiment description	Collection of native mussels near produced water discharge point of Ormen Lange gas processing plant. One reference population compared to two exposed populations.
Target effects	Biomarkers, chemical burdens
Type of data available	Lysosomal stability, micronuclei formation, histochemistry, peroxisomal proliferation, mussel chemistry
Data format	NIVA rapport 5747-2009

Produced water discharge in the North Sea.

Species and life stage	*Mytilus edulis* (farmed), Gadus morhua (farmed)
Exposure time	6 weeks
Exposure concentration	Reference, 500 m, 1000 m, 2000 m, 5000 m, 10 000 m from platform
Experiment description	In situ exposure of Atlantic cod and blue mussel exposed to produced water from oil production platform.
Target effects	Biomarkers
Type of data available	EROD activity, GST activity, Lysosomal stability, DNA adducts, Micronuclei, Histochemistry, Vitellogenin
Data format	Hylland et al., 2008 Mar Poll Bull

2.2.7 Chronic fish field exposure: *Gadhus morhua*

Produced water discharge in the North Sea.

Species and life stage	*Gadus morhua* (farmed)
Exposure time	6 weeks
Exposure concentration	Reference, 500 m, 1000 m, 2000 m, 5000 m, 10 000 m from platform
Experiment description	In situ exposure of Atlantic cod and blue mussel exposed to produced water from oil production platform.
Target effects	Biomarkers
Type of data available	EROD activity, GST activity, Lysosomal stability, DNA adducts, Micronuclei, Histochemistry, Vitellogenin
Data format	Hylland et al., 2008 Mar Poll Bull

2.2.8 *In vitro* systems: Yeast estrogen and androgen screen

YES/YAS: Samples were analysed for estrogenic activity using the yeast estrogen screen (YES), a yeast-based (Saccharomyces cerevisiae) screen developed by Glaxo–Wellcome, plc (Stevenage, Herts, UK), and fully validated by Routledge and Sumpter (1996). The bioassay has been demonstrated to quantitatively detect known estrogens (e.g., 17b-estradiol and estrone) and xenoestrogens (e.g., alkylphenols and Bisphenol A) via a receptor mechanism. The bioassay was carried out using the method of Routledge and Sumpter (1996), with minor variations. Despite not directly relevant to arctic conditions, it could be argued that recombinant yeast screens have importance due to high relevance in determining mechanism of action.. Data exist for two individual PW samples, collected minimum 6 weeks apart. Shown here are results from sampling 1. Both water soluble and particulate fractions of PW were analysed in the yeast screens, but shown here are data from water dissolved fraction only.

Organic extract of water soluble components of produced water from Grane:

Species and life stage	*Saccharomyces cerevisiae*
Exposure time	3 days (YES), 2 days (YAS)
Exposure concentration	Serial dilutions of PW extract
Experiment description	Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS). Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of recombinant yeast. 32 ± 1 ºC
Target effects	Estrogenic ad anti-androgenic effects
Type of data available	Estrogen (E2) equivalents: < 0.03 ng E2/L, Flutamide equivalents: 1578 µg Flut/L
Data format	Tollefsen et al., 2007 Mar Poll Bull

Organic extract of water soluble components of produced water from Gullfaks B:

Species and life stage	*Saccharomyces cerevisiae*
Exposure time	3 days (YES), 2 days (YAS)
Exposure concentration	Serial dilutions of PW extract
Experiment description	Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS). Organic components of

	PW extrated with solid phase extraction, followed by static in vitro exposure of recombinant yeast. 32 ± 1 ºC
Target effects	Estrogenic ad anti-androgenic effects
Type of data available	Estrogen (E2) equivalents: < 0.03 ng E2/L, Flutamide equivalents: 582 µg Flut/L
Data format	Tollefsen et al., 2007 Mar Poll Bull

Organic extract of water soluble components of produced water from Heidrun:

Species and life stage	*Saccharomyces cerevisiae*
Exposure time	3 days (YES), 2 days (YAS)
Exposure concentration	Serial dilutions of PW extract
Experiment description	Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS). Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of recombinant yeast. 32 ± 1 ºC
Target effects	Estrogenic ad anti-androgenic effects
Type of data available	Estrogen (E2) equivalents: < 0.03 ng E2/L, Flutamide equivalents: 1627 µg Flut/L
Data format	Tollefsen et al., 2007 Mar Poll Bull

Organic extract of water soluble components of produced water from Oseberg C:

Species and life stage	*Saccharomyces cerevisiae*
Exposure time	3 days (YES), 2 days (YAS)
Exposure concentration	Serial dilutions of PW extract
Experiment description	Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS). Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of recombinant yeast. 32 ± 1 ºC
Target effects	Estrogenic ad anti-androgenic effects
Type of data available	Estrogen (E2) equivalents: 2.1 ng E2/L, Flutamide equivalents: 3 µg Flut/L
Data format	Tollefsen et al., 2007 Mar Poll Bull

Organic extract of water soluble components of produced water from Snorre A:

Species and life stage	*Saccharomyces cerevisiae*
Exposure time	3 days (YES), 2 days (YAS)
Exposure concentration	Serial dilutions of PW extract
Experiment description	Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS). Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of recombinant yeast. 32 ± 1 ºC
Target effects	Estrogenic ad anti-androgenic effects
Type of data available	Estrogen (E2) equivalents: <0.03 ng E2/L, Flutamide equivalents: 134 µg Flut/L

Data format	Tollefsen et al., 2007 Mar Poll Bull

Organic extract of water soluble components of produced water from Statfjord A:

Species and life stage	*Saccharomyces cerevisiae*
Exposure time	3 days (YES), 2 days (YAS)
Exposure concentration	Serial dilutions of PW extract
Experiment description	Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS). Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of recombinant yeast. 32 ± 1 ºC
Target effects	Estrogenic ad anti-androgenic effects
Type of data available	Estrogen (E2) equivalents: 3.5 ng E2/L, Flutamide equivalents: 430 µg Flut/L
Data format	Tollefsen et al., 2007 Mar Poll Bull

Organic extract of water soluble components of produced water from Statfjord B:

Species and life stage	*Saccharomyces cerevisiae*
Exposure time	3 days (YES), 2 days (YAS)
Exposure concentration	Serial dilutions of PW extract
Experiment description	Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS). Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of recombinant yeast. 32 ± 1 ºC
Target effects	Estrogenic ad anti-androgenic effects
Type of data available	Estrogen (E2) equivalents: - ng E2/L, Flutamide equivalents: - µg Flut/L
Data format	Tollefsen et al., 2007 Mar Poll Bull

Organic extract of water soluble components of produced water from Statfjord C:

Species and life stage	*Saccharomyces cerevisiae*
Exposure time	3 days (YES), 2 days (YAS)
Exposure concentration	Serial dilutions of PW extract
Experiment description	Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS). Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of recombinant yeast. 32 ± 1 ºC
Target effects	Estrogenic ad anti-androgenic effects
Type of data available	Estrogen (E2) equivalents: 4 ng E2/L, Flutamide equivalents: 899 µg Flut/L
Data format	Tollefsen et al., 2007 Mar Poll Bull

Organic extract of water soluble components of produced water from Troll B:

Species and life stage	*Saccharomyces cerevisiae*

Exposure time	3 days (YES), 2 days (YAS)
Exposure concentration	Serial dilutions of PW extract
Experiment description	Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS). Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of recombinant yeast. 32 ± 1 ºC
Target effects	Estrogenic ad anti-androgenic effects
Type of data available	Estrogen (E2) equivalents: 0.4 ng E2/L, Flutamide equivalents: 791 µg Flut/L
Data format	Tollefsen et al., 2007 Mar Poll Bull

Organic extract of water soluble components of produced water from Asgard:

Species and life stage	*Saccharomyces cerevisiae*
Exposure time	3 days (YES), 2 days (YAS)
Exposure concentration	Serial dilutions of PW extract
Experiment description	Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS). Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of recombinant yeast. 32 ± 1 ºC
Target effects	Estrogenic ad anti-androgenic effects
Type of data available	Estrogen (E2) equivalents: 0.1 ng E2/L, Flutamide equivalents: 8171 µg Flut/L
Data format	Tollefsen et al., 2007 Mar Poll Bull

2.2.9 In vitro systems: primary hepatocytes from *Onchorynchus mykiss*

ROS/glutathione/cytotoxicity: The bioassays was carried out using the method of Wang and Joseph, 1999 (ROS), Vandeputte et al., 1994 (glutathione), Schreer et al. 2005 (cytotoxicity). Despite maybe not directly relevant to arctic, it could be argued that *in vitro* analyses of biomarkers in rainbow trout hepatocytes have importance due to high relevance in determining mechanism of action. Data exist for both water soluble and particulate fractions of PW, but shown here are data from water dissolved fraction only, as bioavailability of chemicals in the particulate fraction could be discussed

Organic extract of water soluble components of produced water from Grane:

Species and life stage	*Onchorynchus mykiss*
Exposure time	1 h (ROS formation), 96 h (glutathione, cytotoxicity)
Exposure concentration	0.3, 1, 3, 10, 30, 100, 300 fold concentrated PW
Experiment description	Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of hepatocytes.
Target effects	In vitro biomarkers (oxidative stress and cytotoxicity) in primary cell culture
Type of data available	(LOEC): ROS formation: 3, Glutathione: - , cytotoxicity: 33
Data format	Farmen et al., 2010 Mar Poll Bull

Organic extract of water soluble components of produced water from Troll B:

Species and life stage	Onchorynchus mykiss
Exposure time	1 h (ROS formation), 96 h (glutathione, cytotoxicity)
Exposure concentration	0.3, 1, 3, 10, 30, 100, 300 fold concentrated PW
Experiment description	Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of hepatocytes.
Target effects	In vitro biomarkers (oxidative stress and cytotoxicity) in primary cell culture
Type of data available	(LOEC): ROS formation: 11, Glutathione: 11 , cytotoxicity: 0.3
Data format	Farmen et al., 2010 Mar Poll Bull

Organic extract of water soluble components of produced water from Statfjord C:

Species and life stage	*Onchorynchus mykiss*
Exposure time	1 h (ROS formation), 96 h (glutathione, cytotoxicity)
Exposure concentration	0.3, 1, 3, 10, 30, 100, 300 fold concentrated PW
Experiment description	Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of hepatocytes.
Target effects	In vitro biomarkers (oxidative stress and cytotoxicity) in primary cell culture
Type of data available	(LOEC): ROS formation: 3, Glutathione: 11 , cytotoxicity: 3
Data format	Farmen et al., 2010 Mar Poll Bull

Organic extract of water soluble components of produced water from Statfjord A:

Species and life stage	*Onchorynchus mykiss*
Exposure time	1 h (ROS formation), 96 h (glutathione, cytotoxicity)
Exposure concentration	0.3, 1, 3, 10, 30, 100, 300 fold concentrated PW
Experiment description	Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of hepatocytes.
Target effects	In vitro biomarkers (oxidative stress and cytotoxicity) in primary cell culture
Type of data available	(LOEC): ROS formation: 3, Glutathione: 11 , cytotoxicity: 1
Data format	Farmen et al., 2010 Mar Poll Bull

Organic extract of water soluble components of produced water from Snorre A:

Species and life stage	*Onchorynchus mykiss*
Exposure time	1 h (ROS formation), 96 h (glutathione, cytotoxicity)
Exposure concentration	0.3, 1, 3, 10, 30, 100, 300 fold concentrated PW
Experiment description	Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of hepatocytes.
Target effects	In vitro biomarkers (oxidative stress and

	cytotoxicity) in primary cell culture
Type of data available	(LOEC): ROS formation: 3, Glutathione: 11 , cytotoxicity: 3
Data format	Farmen et al., 2010 Mar Poll Bull

Organic extract of water soluble components of produced water from Gullfaks B:

Species and life stage	*Onchorynchus mykiss*
Exposure time	1 h (ROS formation), 96 h (glutathione, cytotoxicity)
Exposure concentration	0.3, 1, 3, 10, 30, 100, 300 fold concentrated PW
Experiment description	Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of hepatocytes.
Target effects	In vitro biomarkers (oxidative stress and cytotoxicity) in primary cell culture
Type of data available	(LOEC): ROS formation: 3, Glutathione: 33 , cytotoxicity: 110
Data format	Farmen et al., 2010 Mar Poll Bull

Organic extract of water soluble components of produced water from Asgard:

Species and life stage	*Onchorynchus mykiss*
Exposure time	1 h (ROS formation), 96 h (glutathione, cytotoxicity)
Exposure concentration	0.3, 1, 3, 10, 30, 100, 300 fold concentrated PW
Experiment description	Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of hepatocytes.
Target effects	In vitro biomarkers (oxidative stress and cytotoxicity) in primary cell culture
Type of data available	(LOEC): ROS formation: 3, Glutathione: 3 , cytotoxicity: 1
Data format	Farmen et al., 2010 Mar Poll Bull

Organic extract of water soluble components of produced water from Heidrun:

Species and life stage	*Onchorynchus mykiss*
Exposure time	1 h (ROS formation), 96 h (glutathione, cytotoxicity)
Exposure concentration	0.3, 1, 3, 10, 30, 100, 300 fold concentrated PW
Experiment description	Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of hepatocytes.
Target effects	In vitro biomarkers (oxidative stress and cytotoxicity) in primary cell culture
Type of data available	(LOEC): ROS formation: 3, Glutathione: - , cytotoxicity: 3
Data format	Farmen et al., 2010 Mar Poll Bull

Organic extract of water soluble components of produced water from Statfjord B:

Species and life stage	*Onchorynchus mykiss*
Exposure time	1 h (ROS formation), 96 h (glutathione, cytotoxicity)

Exposure concentration	0.3, 1, 3, 10, 30, 100, 300 fold concentrated PW
Experiment description	Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of hepatocytes.
Target effects	In vitro biomarkers (oxidative stress and cytotoxicity) in primary cell culture
Type of data available	(LOEC): ROS formation: 3, Glutathione: - , cytotoxicity: 1
Data format	Farmen et al., 2010 Mar Poll Bull

Organic extract of water soluble components of produced water from Oseberg C:

Species and life stage	*Onchorynchus mykiss*
Exposure time	1 h (ROS formation), 96 h (glutathione, cytotoxicity)
Exposure concentration	0.3, 1, 3, 10, 30, 100, 300 fold concentrated PW
Experiment description	Organic components of PW extrated with solid phase extraction, followed by static in vitro exposure of hepatocytes.
Target effects	In vitro biomarkers (oxidative stress and cytotoxicity) in primary cell culture
Type of data available	(LOEC): ROS formation: 3, Glutathione: - , cytotoxicity: 1
Data format	Farmen et al., 2010 Mar Poll Bull

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