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Exposure levels of alkylphenols causing xenoestrogenic effects in laboratory PW exposed cod, versus realistic levels in field exposed fish

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Contents

Summary

In the present study alkylphenol (AP) exposure data from previous laboratory and field investigations on caged and feral fish were compiled. Supplementary analyses were conducted in order to complete the data matrix and suitable compounds were selected to define a comparable expression of the AP exposure level.

A comparison of AP metabolite levels measured in bile and AP parent compounds measured in liver shows that the metabolite approach is the most sensitive technique for monitoring chronic and low level produced water (PW) exposure. Detectable levels of AP metabolites were not found in bile collected for the 2005 Condition Monitoring.

Based on the investigated material we conclude that the lowest AP exposure levels of PW that gives induction of VTG in the laboratory, are at least ten times higher than the exposure levels present in the proximity (closer than 200 meters) of a typical offshore PW discharge. These findings are in accordance with two previous studies using a model approach and an *in vitro* approach, to investigate the same issue.

Terms and abbreviations

AP	alkylphenol
$C_1 - C_9$	referring to the number of carbons in a side chain
GC-MS	Gas Chromatography – Mass Spectrometry
DMP	Dimethylphenol
E2	Main estrogen fractions: estrone (E1), estradiol (E2), and estriol (E3)
IMR	Institute of Marine Research, Bergen
IRIS	International Research Institute of Stavanger (former RF)
Parent compound	The original form of a compound
Metabolite	Form of a compound after modification by detoxification systems (usually more water soluble than the parent compound)
NOEC	No Observable Effect Concentration
OLF	Oljeindustriens Landsforening, The Norwegian Oil Industry Association
PW	Produced Water
VTG	Vitellogenin (precursor of egg yolk protein)
WCM	Water Column Monitoring
ZRP	Zona radiata protein (egg shell protein)
Xenoestrogens	Compounds that mimic the effect of other estrogens

1 Introduction

Some alkylphenols (APs) have the potential of creating negative reproduction related effects in aquatic organisms like fish (Routledge and Sumpter, 1997). The major toxicological mechanism involved is the potential of these compounds to mimic estrogens and thereby creating feminisation in exposed organisms. The most vital factor for high estrogenic activity of APs is that the alkyl chain is in the *para*-position (*para*>*meta*>*ortho*) and that the chain-length is $\geq C_6$. Maximum activity (400 - 6000 times less potent than E2) has been found for $C_6 - C_9$ *para*-substituted tertiary APs, but *para*-substituted C_5, C_4 and C_3 APs also possess weak estrogenic effects (10^5 - 10^7 times less potent than E2) (Routledge and Sumpter, 1997).

Based on a concern for possible negative reproduction related effects in fish from compounds present in Produced Water (PW) discharges, several laboratory studies have been conducted. Exposure levels that cause negative reproduction related effects in lab, using available methods, have been established (e.g. Meier *et al.* 2002; 2007; Sundt *et al.* 2005, 2007). Common for these studies is that relatively high exposure concentrations of APs have been applied.

From field investigations such as the "*Water Column Monitoring*" projects (Hylland *et al.* 2007; Sundt *et al.* 2006) and the *"Condition Monitoring"* (Grøsvik *et al.* 2007) material suitable for providing exposure estimates were available.

A relevant follow-up was therefore to exploit available information and to obtain new comparable data from available material in order to investigate if exposure levels causing negative effects in lab occur under field conditions. To be able to inter-correlate PW exposure studies, there was a need for a comparable exposure measure. In the present study, a suite of APs was selected and the sum of these was used as a representative expression of the exposure level.

In previous studies involving PW exposure, two different approaches have been applied to investigate the exposure levels of AP to fish. APs have been analysed as parental compounds in liver (e.g. condition monitoring Grøsvik *et al.* 2007) or as metabolites in bile (Sundt *et al.* 2006).

Since this is the first time that results from the two analytical approaches applied on the same material have been available, a validation of the two methods suitability for monitoring of PW discharges have been made.

2 Project objectives

The present project had the following objectives:

1. Compare the overall sensitivity of AP parental compound detection in liver and AP metabolite detection in bile and evaluate the suitability of these two analysis approaches for future monitoring studies.

2. Assess if exposure levels needed to cause xenoestrogenic effects in laboratory exposed fish may occur under field conditions.

To reach these objectives, available AP exposure data and material collected in previous investigations was compiled and supplementary samples were analysed. A comparable expression of AP exposure concentration between studies was defined.

3 Material and methods

The material exploited in the present study was collected for previous investigations where cod was exposed to PW either in laboratory or as part of field investigations. The design of the different studies from where material and AP data are available varies both with respect to the type of exposure compounds (authentic PW or mixes of single compounds) type of exposure (oral or through water) and by duration of the exposures (Table 1).

To mimic PW exposure of fish in the water column, administration through water is considered to be the most realistic way of exposure. The water based exposures are fairly constant over time whereas a diet exposure fluctuates. The delay in absorption of exposure compounds from feed causes complex uptake and excretion kinetics that introduces uncertainty to an experiment if the time between feeding and sampling is not standardised. The less efficient absorption rate in comparison to a water exposure may

also result in unintended low exposure levels if this factor is not taken into consideration.
Another advantage with the use of authentic PW is the possibility to reveal possible effects caused by non AP oil compounds and production chemicals. Due to these physiological and technical limitations, we have in the present study focused on authentic PW exposures when comparing exposure levels.

3.1 Material from previous studies

An overview of the projects that have contributed with material to the present study is given in Table 1.

The low uptake efficiency (approximately 10% of nominal exposure) and the delay in uptake of APs from diet (Sundt *et al. submitted*), limits the uses of the AP metabolite approach as a sensitive exposure marker in laboratory diet exposures. This together with the fact that respiratory uptake is the most realistic way of exposure for fish in the water column led us not to include diet exposures in the comparison.

Table 1 Overview of the material included in the present project. Project name and financing, executing institute, exposure compound and type of exposure, available parameters and duration of exposure is given.

Study (funding)	**Inst.**	**Lab/ field**	**Exposure compound and type of exposure**	**Parameters**	**Exposure duration**
Comparative cod exposure (NFR+)	IRIS/ IMR	lab	Authentic Oseberg C PW Water / AP mix Water	Effect /exposure param.	4 weeks
IMR PW studies (NFR+)	IMR	lab	*PW* Oseberg C *Oral/Water*	*Effect /exposure param.*	*Variable*
WCM biomarker validation "JIP Total and ConocoPhillips"	IRIS	lab	Authentic Ekofisk PW Water, Juvenile fish	Exposure param.	6 weeks
Pre spawning study "JIP Total and ConocoPhillips"	IRIS	lab	Authentic Ekofisk PW Water, Mature fish	Effect /exposure param.	10 weeks
WCM 2006 (OLF)	IRIS/ NIVA	field	Ekofisk PW Water	Exposure param.	6 weeks
Condition monitoring 2005 (OLF)	IMR	field	Feral fish	Exposure param.	Feral fish

3.2 Supplementary material

In order to complete the available data matrix needed for the comparison, supplementary material from the following studies was analyzed:

- Condition monitoring 2005, (financed by OLF, performed by IMR)
  AP metabolites - Sites: Ling bank, Tampen

- Water column monitoring 2006, (financed by OLF, performed by IRIS/NIVA)
  AP parent compounds - Stations: REF 1, station 3 and 4.

- Comparative cod exposure (financed by NFR, performed by IRIS/IMR)
  AP parent compounds – All groups

3.3 Analytical methods

AP parental compounds in liver

The analytical methods employed for extraction and analysis of AP parent compounds in liver tissue is described by Meier *et al.* 2005.

AP metabolites in bile

Methods for extraction and analysis of AP metabolites in bile are described by Jonsson *et al.* 2008.

VTG

Vitellogenin was determined by enzyme linked immuno sorbent assay (ELISA) provided by Biosense Laboratories AS (Bergen, Norway). The analysis was performed either quantitative or semi quantitative according to the manufacturer's instruction.

3.4 Suite of APs for standardised exposure measure

A selection of AP compounds was made in order to provide a standardised parameter for AP exposure levels in the included studies.

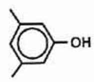
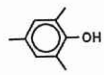
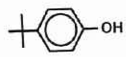
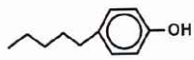
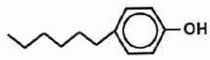
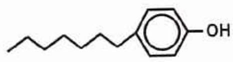
The compounds were selected based on the following criteria:

- Significant levels of the compound present in PW.
- Compounds not present in quantifiable levels in control fish.
- Compounds not present as metabolites from natural physiological processes.
- Significant increased levels observed in laboratory exposed fish.
- Single compound data available from all the studies included.

Some of the selection criteria were based on results from the "pre spawning study" (JIP, Total and ConocoPhillips) presented by Jonsson et al. 2008 and on results from the comparative cod exposure (NFR, Sundt *et al.* in prep). Since the applied parameter serves as a general parameter for exposure, the toxicological potential of the different

AP compounds has not been taken into consideration. The selected compounds are given in Table 2.

Table 2. Alkyl phenols selected for comparison use. Abbreviation, full compound name, CAS no, log octanol-water partition coefficient (log Kow) values of the APs estimated by the *EPA KOWWIN* software version 1.67 and chemical structure is given.

Abbreviation	Compound name	CAS no	log Kow	Structure
3.5-DMP	3.5-dimethylphenol	108-68-9	2.61	
2.4.6-TMP	2.4.6-trimethylphenol	527-60-6	3.15	
4-t-BP	4-tert-butylphenol	98-54-4	3.42	
4-n-PenP	4-n-pentylphenol	14938-35-3	4.02	
4-n- HexP	4-n-hexylphenol	2446-69-7	4.52	
4-n-HepP	4-n-heptylphenol	1987-50-4	5.01	

3.5 Definition of "xenoestrogenic effects" applied in the study.

In the present study we have only considered xenoestrogenic effects in cod; no other possible negative health effects have been addressed. "Xenoestrogenic effect" has been defined as a statistical significant increase of VTG compared to the control group in the experiment. A significant increase in VTG does not necessarily imply that the reproduction is negatively impacted. Increase in VTG is therefore often considered as an early warning signal. In two of the studies (*WCM biomarker validation* and *pre spawning*, Total, ConocoPhillips), ZRP was included as quality assurance for the VTG response.

3.6 Levels of quantification for 3.5-DMP

Significant amounts of 3.5-DMP (*3.5 dimetylphenol*) is present in PW and the compound causes a strong signal when measured in bile if the exposure is sufficiently high. In order to achieve best possible basis of comparison the compound was included in the "AP 6" suite used for evaluation of the analytical approaches. In the samples of feral fish included in the study the levels of APs are below the quantification limit. At such low levels, the analytical background level caused by interference from the deuterated internal standard spike (*3.5 dimetylphenol-D3*) added the samples to provide quantitative estimation exceed the acceptable contribution. 3.5-DMP was therefore not included in the comparison of laboratory and field exposure levels.

4 Results

4.1 Comparison of AP in liver and AP metabolites in bile

In order to evaluate the overall sensitivity of the parent compound and metabolite approaches, data from a common group was compared directly by using data from the *Comparative Cod Exposure* (NFR). For this comparison the most field realistic 1:1000 dilution group was selected. For the available field material, levels of the APs were below the quantification limit and therefore not suitable for comparison.

The present findings show that in fish exposed to a 1:1000 dilution of PW the "6APs" (see definition above) levels in bile measured as deconjugated metabolites are 94 times higher than levels of the same compounds in liver (Figure 1). When measured in higher, which will mean less realistic levels, the difference is even bigger.

Analytically, both the parent compound and metabolite approaches are highly sensitive. But due to the efficient metabolization of light APs by the fish's detoxification systems, the levels of metabolites in bile is higher than parent compounds in liver (Jonsson *et al.* 2007).

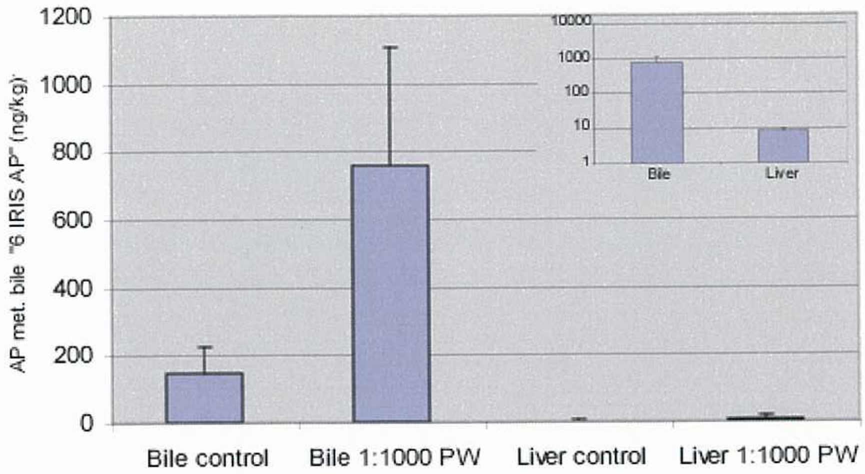


Figure 1. Comparison between “AP 6” (Sum: 3.5-DMP, 2.4.6-TMP, 4-t-BP, 4-n-PenP, 4-n-HexP, 4-n-HepP) levels in bile and liver measured in material from a group of cod exposed to 1:1000 dilution of Oceberg C Produced Water (Comparative cod exposure, NFR). Superimposed chart shows levels in bile and liver of the 1:1000 group presented with a logarithmic scale.

4.2 Comparison of laboratory effect levels with field levels

In the present study effect data (VTG) / AP exposure from three previous laboratory studies were used: *Comparative cod exposure* (NFR), *WCM biomarker validation* (Total, ConocoPhillips) *Pre spawning* (Total, ConocoPhillips), Tollefsen *et al. in prep*, Sundt *et al.* 2005, Sundt *et al.* 2007. AP exposure data given as “5AP” from the highest exposure that did not show VTG induction were used for the comparison (Figure 2). Field exposure levels were available from caged fish collected by the *2006 Water Column Monitoring* (station 3 / 4 pooled and reference 1) and feral fish collected by the *2005 Condition Monitoring* (Ling bank and Tampen).

Based on the investigated material we see that the AP exposure levels needed to cause VTG induction in fish exposed to PW in the laboratory, are at least ten times higher than the exposure levels present in the proximity of a typical offshore discharge (WCM 2006 at Ekofisk, Sundt *et al.* 2006).

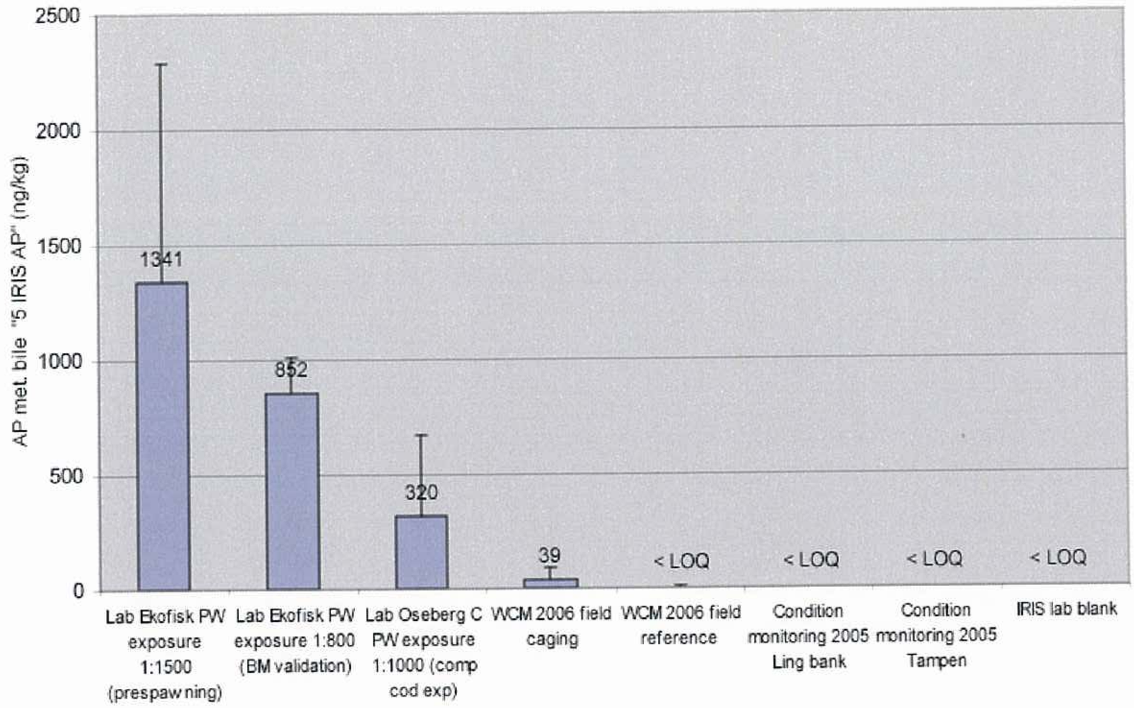


Figure 2. Comparison of "5AP" exposure levels (Sum: 2.4.6-TMP, 4-t-BP, 4-n-PenP, 4-n-HexP, 4-n-HepP) in bile from laboratory exposed groups without xenoestrogenic effects and levels in field material. LOQ: Limit of quantification (15-20 ng/g for single compounds)

The reason why the given NXEL (No Xenoestrogenic Effect Levels) differs among the different PW exposures (*Comparative cod exposure, WCM biomarker validation, Pre spawning*) is due to differences in exposure concentrations and exposure duration applied, together with variance in AP constituents in PWs from different fields (Ekofisk and Oseberg C). AP exposure data are given as "5AP" from the highest exposure not causing measurable xenoestrogenic effects.

4.3 AP exposure levels (metabolite detection) in feral fish

Absence of detectable AP levels in the bile of feral fish collected at the Ling Bank and Tampen area confirm the conclusion from the *2005 Condition monitoring* of levels lower than LOD in these areas (Grøsvik *et al.* 2007).

5 Discussion and conclusions

The present study has relevance for offshore oil field PW monitoring, and in particular for assessing the AP exposure in local populations of fish and for assessing environmental risk of PW contaminants. One important achievement in the study is the development of an approach that makes comparison of exposure levels in fish from different exposures possible.

5.1 Implications for future PW discharges monitoring

The “parent compound / metabolite approach” comparison conducted in the present study shows that levels of AP metabolites in bile are higher than the levels of parent compounds in liver. This is in accordance with previous findings in studies of tissue distribution of the compounds (Tollefsen *et al.* 1998, Sundt *et al. submitted*). It indicates that the metabolite approach provides significantly better sensitivity, a factor that is particularly important for monitoring of chronic and very low exposures typical of diluted PW discharges. Due to the better sensitivity, we suggest that the bile matrix should be used instead of other tissues in future investigations of AP exposure.

5.2 Laboratory exposure levels causing xenoestrogenicity versus field exposure levels

An issue that has been a concern for some time is the possibility that APs pose a threat to the natural reproduction of local fish populations in the proximity of produced water discharges. The present comparison of AP exposure levels indicate that the levels needed to cause xenoestrogenic effects (VTG induction) in fish under laboratory conditions, are at least an order of magnitude higher than levels recorded in field exposed fish.

The highest AP levels measured in field exposed fish included in the present study were the levels found in caged fish from the *2006 Water Column Monitoring*. In this investigation actual PW exposure was confirmed by both AP/PAH levels in cod and PAH levels in mussels from the same stations, compared to a clean reference group (Sundt *et al.* 2006). This caging exposure can be considered as a worst case scenario and we assume that feral fish living in areas affected by PW discharges will be exposed to significantly lower levels of APs. The rationale for this assumption is that feral fish, most likely will move in and out of the area with increased concentration, and hence receive a lower pollution load.

The present finding is in accordance with the conclusions of a study where exposure and effect data were used for modelling the environmental risk (Myhre *et al.* 2006). It is also in accordance with *in vitro* studies of PW estrogenic potential (Tollefsen *et al.* 2006). The fact that three different approaches used to investigate this issue, gives the same answer, increases the scientific certainty.

AP exposure may of course vary with the AP concentrations in the discharged PWs from field to field. The degree of exposure can easily be monitored by measurements of AP metabolites in the bile of fish, and be compared to the effect threshold limits found in the herein referred studies. As the AP metabolite measurement method has been proven to be sufficiently sensitive, reliable and easy to perform, we recommend that this should be done also in the future to safeguard fish populations to PW discharges.

6 Acknowledgements

Total E&P Norge AS and ConocoPhillips Norge are acknowledged for making available unpublished data from the Joint Industry Project “Produced Water effects, Ekofisk case study”.

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Appendix

Raw data.

		3,5-DMP	2,4,6-TMP	4-t-BP	4-n-PenP	4-n-HexP	4-n-HepP	AP6 met sum
Prespawn (bile)								
Control		17,0	9,0	7,0	0,9	1,8	3,0	38,7
Control		21,0	0,3	7,0	0,4	3,0	30,0	61,7
Control		0,8	4,0	11,0	0,0	3,0	23,0	41,8
Control		25,0	0,0	12,0	1,9	8,0	12,0	58,9
Control		22,0	8,0	9,0	0,4	4,0	10,0	53,4
PW 1:1500		103,0	429,0	38,0	13,0	15,0	19,0	617,0
PW 1:1500		257,0	829,0	16,0	32,0	27,0	24,0	1185,0
PW 1:1500		138,0	700,0	14,0	22,0	36,0	21,0	931,0
PW 1:1500		327,0	1828,0	14,0	14,0	24,0	13,0	2220,0
PW 1:1500		283,0	2400,0	12,0	49,0	59,0	58,0	2861,0
PW 1:500		419,0	2435,0	5,0	26,0	39,0	26,0	2950,0
PW 1:500		637,0	2918,0	10,0	81,0	91,0	70,0	3807,0
PW 1:500		372,0	2025,0	14,0	69,0	89,0	59,0	2628,0
PW 1:500		375,0	2323,0	7,0	48,0	65,0	45,0	2863,0
PW 1:500		317,0	1970,0	15,0	73,0	80,0	63,0	2518,0
BM validation	**(bile)**							
1:800		257,0	745,0	6,9	19,0	13,8	6,9	1048,6
1:800		408,0	898,0	3,6	29,0	11,2	5,8	1355,6
1:800		381,0	761,0	7,4	26,0	14,2	7,8	1197,4
WCM 2006 (liver)								
Reference	129	0,62	0,01	0,08	0,05	0,02	0,00	0,01
Reference	130	0,11	0,00	0,05	0,01	0,01	0,00	0,00
Station 3	322	0,09	0,02	0,06	0,01	0,02	0,01	0,02
Station 3	328	0,08	0,02	0,04	0,00	0,00	0,00	0,02
Station 3	329	0,11	0,03	0,05	0,01	0,00	0,00	0,03
Station 3	330	0,36	0,10	0,12	0,03	0,08	0,00	0,10
Station 4	412	0,13	0,04	0,04	0,01	0,01	0,00	0,04
Station 4	429	0,09	0,02	0,05	0,01	0,00	0,00	0,02
Station 4	430	0,07	0,01	0,05	0,01	0,01	0,00	0,01
blank	blank	0,08	0,01	0,06	0,00	0,00	0,00	0,01
WCM 2006 (bile)								
Reference	111	30,6	0,0	0,0	0,0	0,0	0,0	30,6
Reference	114	32,6	0,0	0,0	0,0	0,0	0,0	32,6
Reference	115	30,7	0,0	0,0	0,0	0,0	0,0	30,7
Reference	116	23,0	0,0	0,0	0,0	0,0	0,0	23,0
Reference	117	58,5	0,0	0,0	0,0	0,0	0,0	58,5
Reference	119	25,6	0,0	0,0	0,0	0,0	0,0	25,6
Reference	120	25,1	0,0	0,0	0,0	0,0	0,0	25,1
Reference	121	28,5	0,0	0,0	0,0	0,0	0,0	28,5
Reference	122	27,5	0,0	0,0	0,0	0,0	0,0	27,5
Reference	123	25,0	0,0	0,0	0,0	0,0	0,0	25,0
Reference	124	26,0	0,0	0,0	0,0	0,0	0,0	26,0

Reference	125	21,0	0,0	0,0	0,0	0,0	0,0	21,0
Reference	126	28,0	0,0	0,0	0,0	0,0	0,0	28,0
St 3	311	53,7	51,9	0,0	0,0	0,0	0,0	105,6
St 3	312	71,5	70,0	0,0	0,0	0,0	30,0	171,5
St 3	313	73,3	50,2	0,0	0,0	0,0	0,0	123,4
St 3	314	50,7	27,6	0,0	0,0	0,0	0,0	78,3
St 3	315	43,9	33,6	41,0	0,0	0,0	0,0	118,5
St 3	316	51,3	0,0	0,0	0,0	0,0	0,0	51,3
St 3	317	39,8	0,0	0,0	0,0	0,0	0,0	39,8
St 3	318	59,1	0,0	0,0	0,0	21,0	0,0	80,1
St 3	319	30,7	0,0	37,8	0,0	0,0	0,0	68,5
St 3	320	37,4	0,0	0,0	0,0	0,0	0,0	37,4
St 3	321	52,4	58,6	0,0	0,0	0,0	0,0	111,0
St 3	322	82,0	144,6	0,0	0,0	0,0	21,0	247,6
St 3	323	42,6	0,0	0,0	0,0	0,0	0,0	42,6
St 3	324	64,1	52,7	0,0	0,0	0,0	0,0	116,8
St 3	325	56,8	57,9	0,0	0,0	0,0	25,0	139,7
St 3	326	86,5	90,6	0,0	0,0	0,0	0,0	177,1
ST 4	411	72,5	0,0	0,0	0,0	0,0	33,0	105,5
ST 4	412	53,1	57,0	0,0	0,0	0,0	32,0	142,1
ST 4	413	52,9	35,3	0,0	0,0	0,0	32,0	120,2
ST 4	414	64,0	18,9	0,0	0,0	0,0	24,0	106,9
ST 4	415	55,6	0,0	0,0	0,0	0,0	0,0	55,6
ST 4	416	49,5	0,0	0,0	0,0	0,0	0,0	49,5
ST 4	417	33,7	0,0	0,0	0,0	0,0	0,0	33,7
ST 4	418	48,6	0,0	0,0	0,0	0,0	0,0	48,6
ST 4	419	74,7	54,4	0,0	0,0	0,0	0,0	129,1
ST 4	420	41,8	0,0	0,0	0,0	0,0	0,0	41,8
ST 4	421	65,3	78,5	0,0	0,0	0,0	0,0	143,8
ST 4	422	46,4	28,7	0,0	0,0	0,0	0,0	75,1
ST 4	423	47,3	36,4	0,0	0,0	0,0	0,0	83,7
ST 4	424	47,1	42,4	0,0	0,0	0,0	0,0	89,5
ST 4	425	42,0	0,0	0,0	0,0	0,0	0,0	42,0
ST 4	427	39,3	0,0	0,0	0,0	0,0	0,0	39,3
ST 4	428	84,6	0,0	0,0	0,0	0,0	0,0	84,6
IMR diet								
4000ppbC4-7	1	0,0	0,0	0,0	55,4	345,4	224,2	625,0
4000ppbC4-7	2	0,0	0,0	0,0	28,1	176,0	111,8	315,9
4000ppbC4-7	3	0,0	0,0	0,0	50,1	274,2	180,4	504,7
4000ppbC4-7	4	0,0	0,0	57,3	33,5	222,4	179,9	493,1
20ppbC4-7	1	0,0	0,0	0,0	32,0	79,8	73,4	185,3
20ppbC4-7	2	0,0	0,0	0,0	24,5	65,5	46,7	136,7
20ppbC4-7	3	0,0	0,0	0,0	26,3	56,0	44,4	126,7
20ppbC4-7	4	0,0	0,0	0,0	22,5	47,1	51,1	120,7
20ppbC4-7	5	0,0	0,0	0,0	20,5	44,5	55,2	120,2
Comp cod exp								
Control	S1 - 020605	0,7	8,9	113,5	7,9	3,9	16,5	151,4
Control	S11 - 020605	20,1	5,8	119,4	15,3	44,6	17,9	223,1
Control	S4 - 030605	9,4	1,5	105,6	8,1	37,9	17,1	179,6
Control	S13 - 030605	34,2	1,8	124,2	13,9	11,3	43,1	228,5
Control	S8 - 060605	25,0	16,8	125,1	7,7	16,0	17,3	207,9
Control	S1 - 070605	54,1	0,8	10,5	7,0	11,8	21,9	106,1

Control	S8 - 070605	20,4	20,8	110,7	16,4	37,4	60,6	266,3
Control	S1 - 090605	18,1	2,1	3,9	8,6	20,5	23,0	76,2
Control	S1 - 100605	19,2	1,5	7,8	6,7	13,0	24,9	73,1
Control	S1 - 110605	17,0	0,9	3,8	3,2	4,5	9,2	38,6
Control	S17 - 110605	16,1	2,9	5,5	6,4	20,2	27,6	78,7
Spiked control	S2 - 020605	3482,4	3208,7	2728,1	3003,3	3225,7	2334,9	17983,1
Spiked control	S2 - 020605	3491,9	3131,9	2808,5	3190,5	3410,7	2475,0	18508,5
Spiked control	S2 - 060605	3950,7	3454,7	3166,6	3564,2	3748,0	2797,7	20681,9
Spiked control	S2 - 070605	3478,7	3086,8	2739,6	3202,4	3416,7	2523,4	18447,6
Spiked control	S2 - 060605	3910,0	3378,6	3261,0	3579,7	3760,8	2792,3	20682,4
Spiked control	S2 - 070605	3456,1	3139,0	2786,6	3305,0	3442,8	2540,8	18670,3
Spiked control	S2 - 020605	3417,9	3099,0	2914,3	3221,7	3477,4	2531,4	18661,7
Spiked control	S2 - 090605	1682,3	1579,0	1415,6	1580,4	1638,9	1206,0	9102,2
Spiked control	S2 - 100605	3508,1	3279,4	2869,7	3356,1	3569,1	2593,5	19175,9
Spiked control	S2 - 110605	1606,1	1523,7	1289,4	1485,0	1560,3	1121,5	8586,0
Spiked control	S18 - 110605	854,1	781,0	681,3	743,5	800,6	596,4	4456,9
C4-C7 low	S3 - 020605	7,6	5,9	128,7	89,9	390,7	304,3	927,1
C4-C7 low	S12 - 020605	52,7	23,9	154,6	124,0	397,5	309,2	1061,9
C4-C7 low	S5 - 030605	9,7	15,6	148,6	127,2	547,0	429,0	1277,1
C4-C7 low	S9 - 060605	8,4	8,5	137,4	101,0	417,3	362,6	1035,2
C4-C7 low	S14 - 060605	30,6	9,1	150,0	139,8	557,3	492,2	1379,0
C4-C7 low	S9 - 070605	43,7	10,7	146,7	190,0	653,3	613,7	1658,1
C4-C7 low	S3 - 090605	25,5	13,1	20,7	97,3	385,9	316,2	858,7
C4-C7 med	S4 - 020605	16,8	16,0	162,3	540,8	2326,7	1776,9	4839,5
C4-C7 med	S13 - 020605	13,6	15,0	193,0	601,7	27,3	2399,0	3249,6
C4-C7 med	S6 - 030605	15,4	9,8	185,0	571,2	2195,9	1856,9	4834,2
C4-C7 med	S10 - 060605	27,9	7,7	165,1	490,8	2220,9	1670,4	4582,8
C4-C7 med	S15 - 060605	37,4	17,3	303,7	738,1	3342,9	2463,0	6902,4
C4-C7 med	S10 - 070605	44,5	10,9	197,4	697,3	2960,9	2624,4	6535,4
C4-C7 med	S4 - 090605	17,4	3,6	49,5	579,4	2301,4	1881,5	4832,8
C4-C7 high	S5 - 020605	55,9	14,7	20906,8	173836,6	347243,7	286538,5	828596,2
C4-C7 high	S14 - 020605	66,7	16,2	10532,8	58888,1	266719,4	209528,6	545751,8
C4-C7 high	S7 - 030605	49,9	29,3	11666,6	140904,2	354536,0	284046,9	791232,9
C4-C7 high	S11 - 060605	71,3	16,3	7547,6	94329,3	399467,3	323738,3	825170,1
C4-C7 high	S16 - 060605	56,2	31,5	16035,5	121727,3	450248,6	351948,4	940047,5
C4-C7 high	S11 - 070605	100,7	18,6	25793,7	114880,5	418219,4	332110,3	891123,2
C4-C7 high	S5 - 090605	55,4	7,1	16098,4	105700,7	399010,9	335755,4	856627,9
PW 1:1000	S6 - 020605	858,4	135,5	115,1	22,7	16,7	31,0	1179,4
PW 1:1000	S15 - 020605	860,8	218,8	123,4	22,5	29,5	37,9	1292,9
PW 1:1000	S8 - 030605	231,0	53,7	111,0	17,8	36,1	24,2	473,8
PW 1:1000	S3 - 060605	198,6	42,2	124,4	10,6	12,3	12,9	401,0
PW 1:1000	S12 - 060605	227,9	92,9	134,4	20,1	32,1	32,5	539,9
PW 1:1000	S3 - 070605	289,1	155,8	181,3	17,5	28,3	22,2	694,2
PW 1:1000	S12 - 070605	380,9	139,5	130,8	17,0	14,3	20,6	703,1
PW 1:200	S16 - 020605	1312,4	245,1	117,4	63,0	18,5	25,6	1782,0
PW 1:200	S9 - 030605	1442,3	655,7	133,0	66,7	63,1	50,0	2410,8
PW 1:200	S7 - 020605	2159,8	791,1	127,3	82,8	44,4	32,1	3237,5
PW 1:200	S4 - 060605	1978,4	569,4	160,2	30,2	19,0	17,4	2774,6
PW 1:200	S13 - 060605	2402,6	675,6	138,2	89,6	72,6	33,4	3412,0
PW 1:200	S4 - 070605	2587,6	840,7	1725,4	69,2	85,7	32,9	5341,5
PW 1:200	S13 - 070605	8746,9	901,9	249,1	61,5	44,7	49,1	10053,2
AP mix 9	S8 - 020605	477,9	4050,4	1348,8	4793,0	5199,8	2545,6	18415,5

AP mix 9	S1 - 030605	1896,8	4133,4	1545,6	2878,3	4084,3	1916,2	16454,6
AP mix 9	S10 - 030605	610,9	4458,6	1407,9	6917,5	7846,7	3695,3	24936,9
AP mix 9	S14 - 030605	822,9	4599,3	3108,4	7745,1	9534,4	4958,4	30768,5
AP mix 9	S5 - 060605	779,9	3878,5	1485,2	9332,8	14090,0	6193,3	35759,7
AP mix 9	S5 - 070605	474,3	2937,6	536,5	5049,2	7574,4	3776,6	20348,6
AP mix 9	S14 - 070605	443,9	2962,5	635,3	2942,6	3095,0	1625,0	11704,3
Oil spike	S9 - 020605	1929,6	8688,6	3636,8	8838,1	13188,9	5175,7	41457,7
Oil spike	S2 - 030605	1416,9	11868,6	2609,4	10511,7	12621,3	6043,6	45071,5
Oil spike	S11 - 030605	1719,1	11449,1	2318,6	10301,2	12961,0	5350,2	44099,2
Oil spike	S6 - 060605	2343,4	11482,4	5557,4	17851,6	21906,3	8756,4	67897,5
Oil spike	S6 - 070605	2082,9	10069,5	3886,9	14336,2	19913,0	7725,6	58014,1
Oil spike	S15 - 070605	1971,9	11349,3	2099,0	14840,0	17443,0	7929,5	55632,7
Oil	S10 - 020605	20,2	30,9	125,0	12,4	37,8	78,6	304,9
Oil	S3 - 030605	52,8	35,5	103,9	10,3	24,3	43,7	270,5
Oil	S12 - 030605	34,5	118,6	115,8	13,6	44,4	60,6	387,5
Oil	S7 - 060605	35,9	39,0	131,6	11,5	63,7	73,1	354,8
Oil	S7 - 070605	50,7	34,7	121,6	22,0	105,5	109,1	443,6
Oil	S16 - 070605	23,5	68,0	112,2	23,2	70,7	101,1	398,7
Comp cod exp (liver)								
Control	5297	0,3	0,0	0,5	0,2	0,1	1,2	2,3
Control	5636	1,0	0,0	1,7	1,0	0,2	1,7	5,6
Control	5AED	0,4	0,0	0,6	0,1	0,1	0,3	1,4
Control	A693	0,4	0,0	0,6	0,1	0,1	1,2	2,4
Control	A2D8	0,6	0,0	0,4	0,2	0,1	1,2	2,6
Control	97FC	0,4	0,0	0,6	0,1	0,1	0,8	1,9
Control	A2FC	0,4	0,0	0,5	0,1	0,0	0,5	1,5
Control	9773	0,5	0,0	0,6	0,7	0,2	0,0	1,9
Control	9700	0,2	0,1	0,5	0,2	0,1	1,0	2,1
C4-C7 low	9,5E+10	0,8	0,0	2,0	0,7	0,4	2,3	6,1
C4-C7 low	2AE9	0,8	0,0	0,6	0,5	0,3	1,7	3,9
C4-C7 low	2EFA	0,5	0,0	0,8	0,3	0,2	0,9	2,8
C4-C7 low	91FA	0,7	0,0	0,9	0,8	0,2	2,4	5,1
C4-C7 low	62BA	0,4	0,0	0,5	0,4	0,2	1,9	3,3
C4-C7 low	A121	0,7	0,0	0,9	0,5	0,5	1,4	4,0
C4-C7 medium	A64B	1,1	0,0	1,3	1,1	0,6	3,2	7,4
C4-C7 medium	3731	1,1	0,0	2,9	1,7	0,9	3,8	10,4
C4-C7 medium	A135	0,4	0,0	3,0	2,7	1,8	10,6	18,5
C4-C7 medium	964A	0,5	0,0	0,9	1,1	0,3	2,5	5,3
C4-C7 medium	41CA	0,6	0,1	0,9	0,8	0,3	3,0	5,7
C4-C7 medium	8AFC	0,8	0,0	1,0	1,1	0,6	3,4	6,8
C4-C7 medium	6CC2	0,7	0,0	1,7	1,4	0,8	4,4	9,0
PW 1:1000	47E0	9,4	0,1	3,6	1,3	0,8	4,7	19,9
PW 1:1000	3F7B	8,8	0,1	0,7	0,4	0,2	0,8	11,1
PW 1:1000	588E	1,0	0,0	0,9	0,2	0,2	0,8	3,1
PW 1:1000	55DF	0,4	0,0	1,0	0,8	0,5	0,9	3,5
PW 1:1000	3253	0,5	0,0	0,6	0,2	0,2	1,1	2,6
PW 1:200	4997	1,6	0,2	2,1	0,6	0,3	2,6	7,3
PW 1:200	56E4	0,7	0,0	0,9	0,4	0,2	0,9	3,0
PW 1:200	4529	1,9	0,2	2,2	0,6	0,4	2,3	7,6
PW 1:200	2FBB	0,9	0,0	0,5	0,2	0,1	0,8	2,6
PW 1:200	5365	0,8	0,0	0,7	0,4	0,2	0,3	2,4
AP 9 mix	354E	2,7	0,1	10,0	7,2	0,8	3,2	24,0

AP 9 mix	62C5	48,8	0,7	47,6	29,4	3,4	11,5	141,4
AP 9 mix	4E11	0,5	0,2	9,5	15,3	1,6	6,5	33,6
AP 9 mix	477C	1,2	0,1	20,4	21,8	2,4	9,2	55,2
AP 9 mix	4F9E	0,9	0,7	13,9	31,7	6,1	13,4	66,7
AP 9 mix	6CFB	0,5	0,0	7,1	14,8	3,3	6,6	32,3
AP 9 mix	3E47	0,5	0,1	18,6	12,5	2,0	6,1	39,8
AP 9 mix	540D	1,3	0,0	8,6	14,0	2,2	6,1	32,2
AP 9 mix	5688	0,8	0,1	19,2	23,7	2,7	10,0	56,6
Oil+AP+PAH	6FF7	1,5	0,2	23,0	22,9	2,6	10,4	60,6
Oil+AP+PAH	7C48	1,0	0,3	15,8	27,2	5,6	16,2	66,1
Oil+AP+PAH	5B62	0,9	0,0	13,6	18,0	3,9	11,2	47,6
Oil+AP+PAH	424B	1,4	0,3	8,9	40,1	5,0	25,0	80,6
Oil+AP+PAH	42F2	0,8	0,0	9,8	10,8	1,9	6,4	29,7
Oil+AP+PAH	78CC	0,6	0,2	13,7	26,5	1,8	11,8	54,5
Oil+AP+PAH	4EDD	0,3	0,1	40,0	33,3	5,9	19,2	98,9
Oil	8B24	0,6	0,1	0,7	1,0	0,5	3,3	6,2
Oil	5DD1	0,2	0,0	0,5	0,4	0,2	0,9	2,2
Oil	5E2A	0,3	0,0	0,4	0,8	1,2	0,0	2,6
Oil	52B1	0,4	0,1	0,6	0,9	0,3	1,5	3,7
Oil	82FF	0,2	0,0	0,4	0,2	0,1	1,3	2,3
Condition monitoring 2005 (liver)								
Ling bank	Group average	1,0	0,0	1,0	0,2	3,0	0,2	5,4
Tampen	Group average	0,7	0,0	0,6	0,1	2,0	0,1	3,5
Condition monitoring 2005 (bile)								
Ling bank	LB 8	45,0	0,0	0,0	0,0	0,0	0,0	45,0
Ling bank	LB 15	45,0	0,0	0,0	0,0	0,0	0,0	45,0
Ling bank	LB 21	49,0	0,0	0,0	0,0	0,0	0,0	49,0
Ling bank	LB 22	49,0	0,0	0,0	0,0	0,0	0,0	49,0
Ling bank	LB 23	50,0	0,0	0,0	0,0	0,0	0,0	50,0
Ling bank	LB 25	51,0	0,0	0,0	0,0	0,0	0,0	51,0
Ling bank	LB 28	47,0	0,0	0,0	0,0	0,0	0,0	47,0
Tampen	Ta 12	45,0	0,0	0,0	0,0	0,0	0,0	45,0
Tampen	Ta 13	45,0	0,0	0,0	0,0	0,0	0,0	45,0
Tampen	Ta 14	50,0	0,0	0,0	0,0	0,0	0,0	50,0
Tampen	Ta 15	51,0	0,0	0,0	0,0	0,0	0,0	51,0
Tampen	Ta 20	51,0	0,0	0,0	0,0	0,0	0,0	51,0
Tampen	Ta 21	48,0	0,0	0,0	0,0	0,0	0,0	48,0
Tampen	Ta 22	50,0	0,0	0,0	0,0	0,0	0,0	50,0
Tampen	Ta 23	51,0	0,0	0,0	0,0	0,0	0,0	51,0
Tampen	Ta 25	50,0	0,0	0,0	0,0	0,0	0,0	50,0
IRIS lab control								
Lab control		49,0	0,0	0,0	0,0	0,0	0,0	49,0
Lab control		53,0	0,0	0,0	0,0	0,0	0,0	53,0