

The CONffIDENCE project - The outcome of WP3 on heavy metals

Sloth, Jens Jørgen

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Work package 3

Heavy metals

CONffIDENCE

Stakeholder workshop

Brussels 20. September 2012

DTU Food
National Food Institute

WP leader
Jens J. Sloth

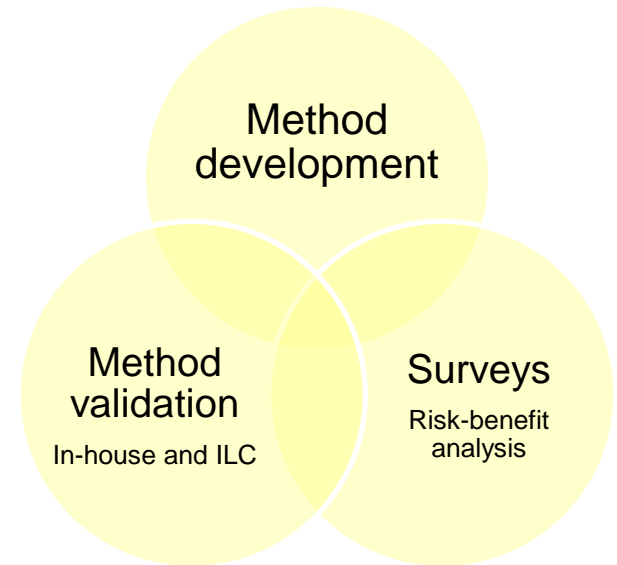


www.confidence.eu



Agenda

- **The CONffIDENCE project** – general information
- **WP3 on "heavy metals"** – in focus
- **Inorganic arsenic**
 - SPE HG-AAS method
 - seafood samples
 - rice samples
- **Methylmercury**
 - HPLC-ICPMS method
 - seafood samples
 - feed samples



CONFIDENCE in a nutshell

CONtaminants in **FOOD** and **FEED** – Inexpensive **DE**tectio**N** for **C**ontrol of **E**xposure

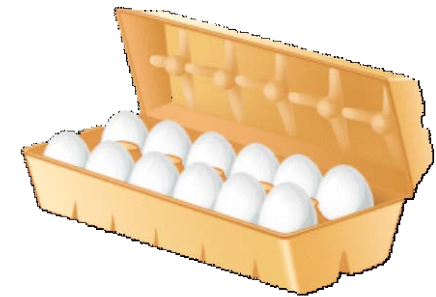
- Collaborative Project: FP7 (European Commission)
- Duration: May 2008 – Dec 2012
- 16 partners from 10 countries, representing universities, research institutes, industry and SMEs
- Budget: 7.5 Mio €
- Coordinator: RIKILT - Institute of Food Safety, part of Wageningen UR (NL)
- WP3 leader: DTU Food



The commodities

➤ Food & Feed

- Fish/shellfish and fish feed
- Cereals and cereal-based feed
- Potatoes/vegetables
- Honey
- Eggs
- Meat
- Dairy products



The target contaminants

- POPs:
 - dioxin-like PCBs + metabolites
 - brominated flame retardants
 - polycyclic aromatic hydrocarbons (PAH)
- Perfluorinated compounds (PFCs)
- Pesticides: paraquat/diquat, dithiocarbamates
- Veterinary drugs:
 - antibiotics, e.g. tetracyclines
 - coccidiostats, e.g. ionophores
- **Heavy metals speciation:**
 - inorganic arsenic
 - methylmercury
- Biotoxins:
 - alkaloids
 - marine biotoxins
 - mycotoxins



CONFIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

CONFIDENCE project objectives

CONFIDENCE is an ambitious project, which aims to further expand Europe's excellent position in (i) food safety issues and (ii) chemical detection technology, as well as to ensure the competitiveness of the involved European industries. The project has five major objectives:

- Assurance of quality and safety in the European food supply from farm to fork by the development of new simplified detection methods for chemical contaminants with effective features: fast, easy-to-use, robust, high-throughput, broad-spectrum (multiplex) and cost-efficient
- Development of new detection tools for key- and emerging risks as recognised by the European Food Safety Agency (EFSA), e.g. perfluorinated compounds and naturally occurring toxins from algae, plants and fungi;
- Improvement of consumer exposure assessments. The developed fast and cost-efficient methods will allow a higher sampling and analysis density in monitoring. Thus, a better understanding of contaminant levels in food and feed will be achieved;
- Contribution to the validation of risk-benefit and predictive hazard behaviour models in accordance with the strategic agenda of the European Technology Platform (ETP) Food for Life;
- Extensive dissemination and training of new detection methods to all relevant stakeholders, including industrial and governmental end-users and students, to advance technology exploitation.

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EVENT

20 Sept 2012
CONFIDENCE CLUSTER 3
WORKSHOP AND EURL-
HEAVY METALS ANNUAL
MEETING

07 - 10 Oct 2012
7TH EUROPEAN
CONFERENCE ON
PESTICIDES AND RELATED
ORGANIC
MICROPOLLUTANTS IN THE
ENVIRONMENT AND 13TH
SYMPOSIUM ON CHEMISTRY
AND FATE OF MODERN
PESTICIDES

24 - 26 Oct 2012
INTERNATIONAL MPU
WORKSHOP 2012: PLANT
PROTECTION FOR THE



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CONFIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure



CONFIDENCE NEWS

May 2012 - Issue 8

[In the spotlight](#)

[News from the CONFIDENCE project](#)

[News from other projects](#)

[Upcoming Events](#)

Dear stakeholder,

The CONFIDENCE project team is proud to present the 8th edition of the CONFIDENCE e-newsletter. In this newsletter you will find recent developments in the CONFIDENCE project and related information in the area of contaminants in food and feed.

Newsletter –
2 times/year



WP3 overall objectives

Objectives

Development of simplified methodologies for the determination of

- 1) **inorganic arsenic (iAs)** in seafood
- 2) **methylmercury (MeHg)** in marine based food and feed.

2 parallel approaches were followed

- 1) **cytosensor** approach using luminescent bacterial cell biosensor
- 2) **solid phase extraction** approach followed by AAS (SPE-AAS)



WP3 - relevance

➤ Current situation in EU legislation:

Foodstuffs

MLs for Pb, Cd, Hg and Sn
EU directive 2006/1881/EC (and amendments)

Animal feedingstuffs

MLs for As, Pb, Cd and Hg
EU directive 2002/32/EC (and amendments)

**Only maximum levels for
total concentration of the metals**

Arsenic

- inorganic As (iAs) is the toxic form of As
- Lack of specific data on iAs (*EFSA, 2009 and JECFA, 2010*)
- Lack of validated, standardised methods (*EFSA, JECFA*)

Mercury

- Methylmercury is considered more toxic than inorganic Hg (iHg)

Seafood/marine feed

- Seafood is the predominant source of As and Hg in the European diet
- Focus on marine feed and food sample types



EFSA (2009) and JECFA (2010) opinions on arsenic in food

- Old PTWI value (WHO, 1988) was withdrawn
- **NEW!** $BMDL_{1.0} = 0.3 - 8 \mu\text{g/kg bw per day}$ for inorganic arsenic
- => EU dietary exposures within this range
- => Risk to some consumers cannot be excluded
- **NEW!** $BMDL_{0.5} = \underline{3 \mu\text{g/kg bw per day}}$ for inorganic arsenic
- => *0.5% increased incidence of lung cancer for 12 y exposure*

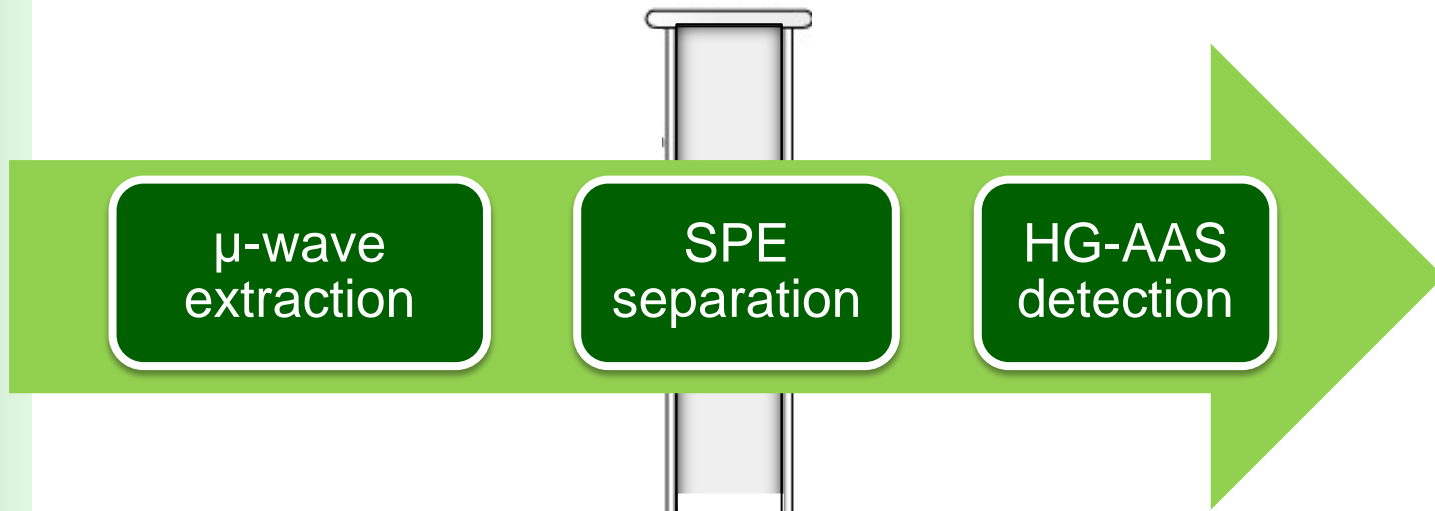


- “...there is a need to produce speciation data for different food commodities to support dietary exposure assessment...”
- “...more accurate information on the inorganic arsenic content of foods is needed to improve assessments of dietary exposures to inorganic arsenic”
- “...need for validated methods for selective determination of inorganic arsenic in food matrices”

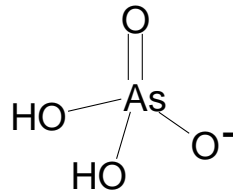


Arsenic speciation analysis

speciation alternative: SPE, HG-AAS



hydride generation
atomic absorption
spectrometry



inorganic arsenic



μ -wave extraction - oxidation of As(III) to As(V)

0.2 g sample
+ 10 mL extractant
(0.06 M HCl, 3% H₂O₂)

25 minutes at 90°C

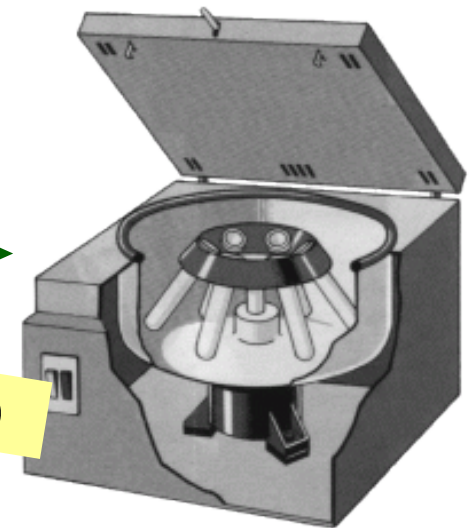
Centrifugation
10 min 2100 x g



Glas vessel



μ -wave oven

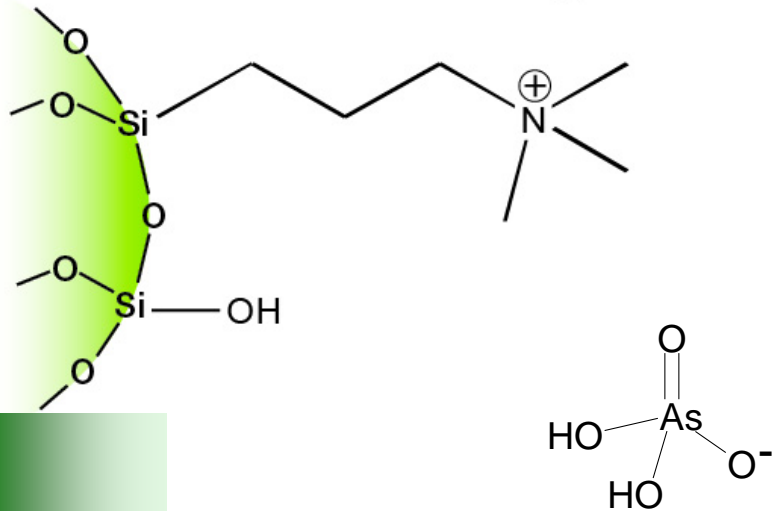


centrifuge

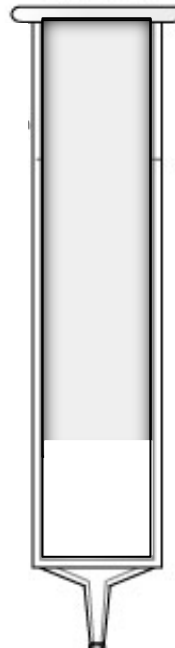
Quantitative conversion: As(III) \rightarrow As(V)



SPE protocol - separation of As species



Strong anion exchange SPE column
silica based
Strata SAX
500 mg/6 mL, Phenomenex



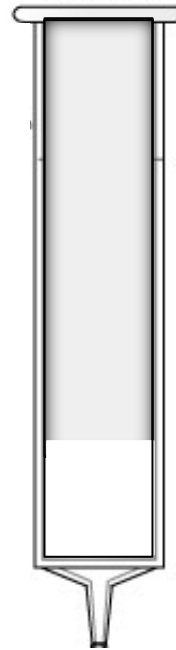
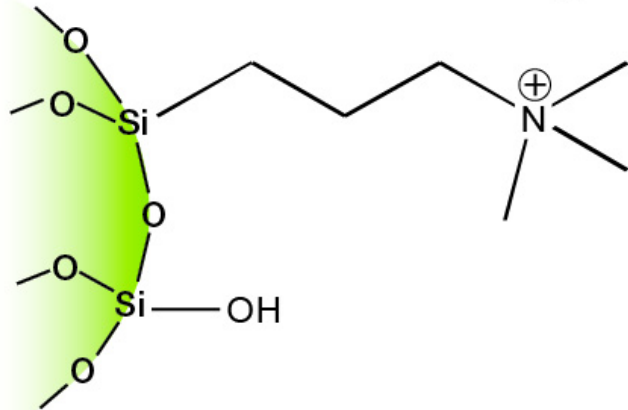
The **charge** of the arsenic species depends on pH

@ pH = 6 **iAs(V)** is **negatively charged**

Sequential elution
Separation of inorganic As from organo As species by SPE



SPE protocol - Separation of As species



Condition

100 % MeOH

Equilibrate

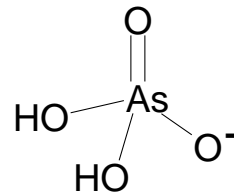
Buffer: 20mM $(\text{NH}_4)_2\text{CO}_3$, 0.03 M HCl and 1.5% H_2O_2

Load

Buffered sample: pH 5.0-7.5

Wash 0.5 M CH_3COOH

Elute 0.5 M HCl



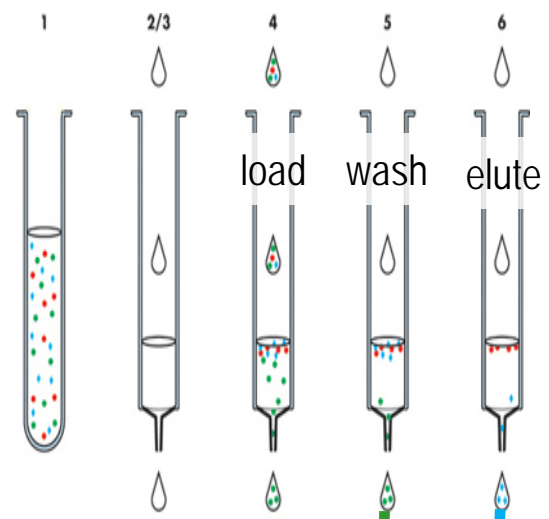
SPE-HG-AAS – a novel speciation alternative...

μ -wave extraction

Separation by SPE

Detection by HG-AAS

Inexpensive detection system



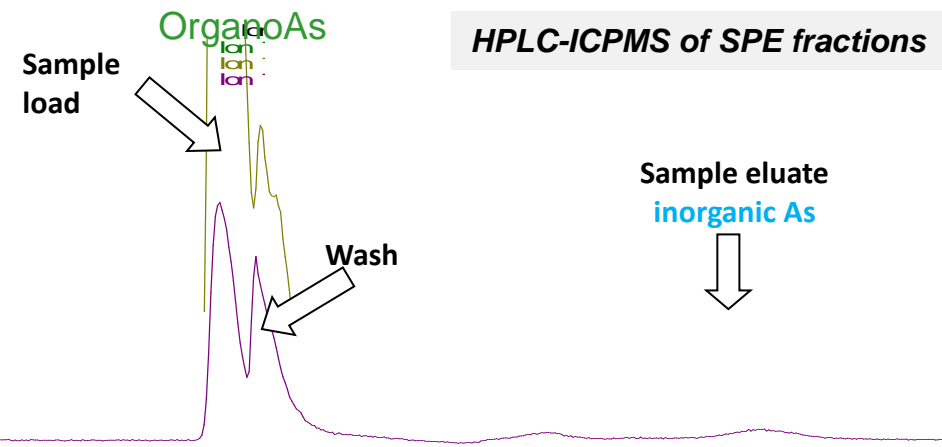
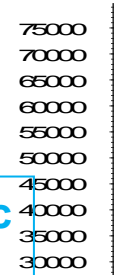
Sequential elution for selective off-line separation of inorg As from organo As species by SPE

ANALYTE * INTERFERENCES **

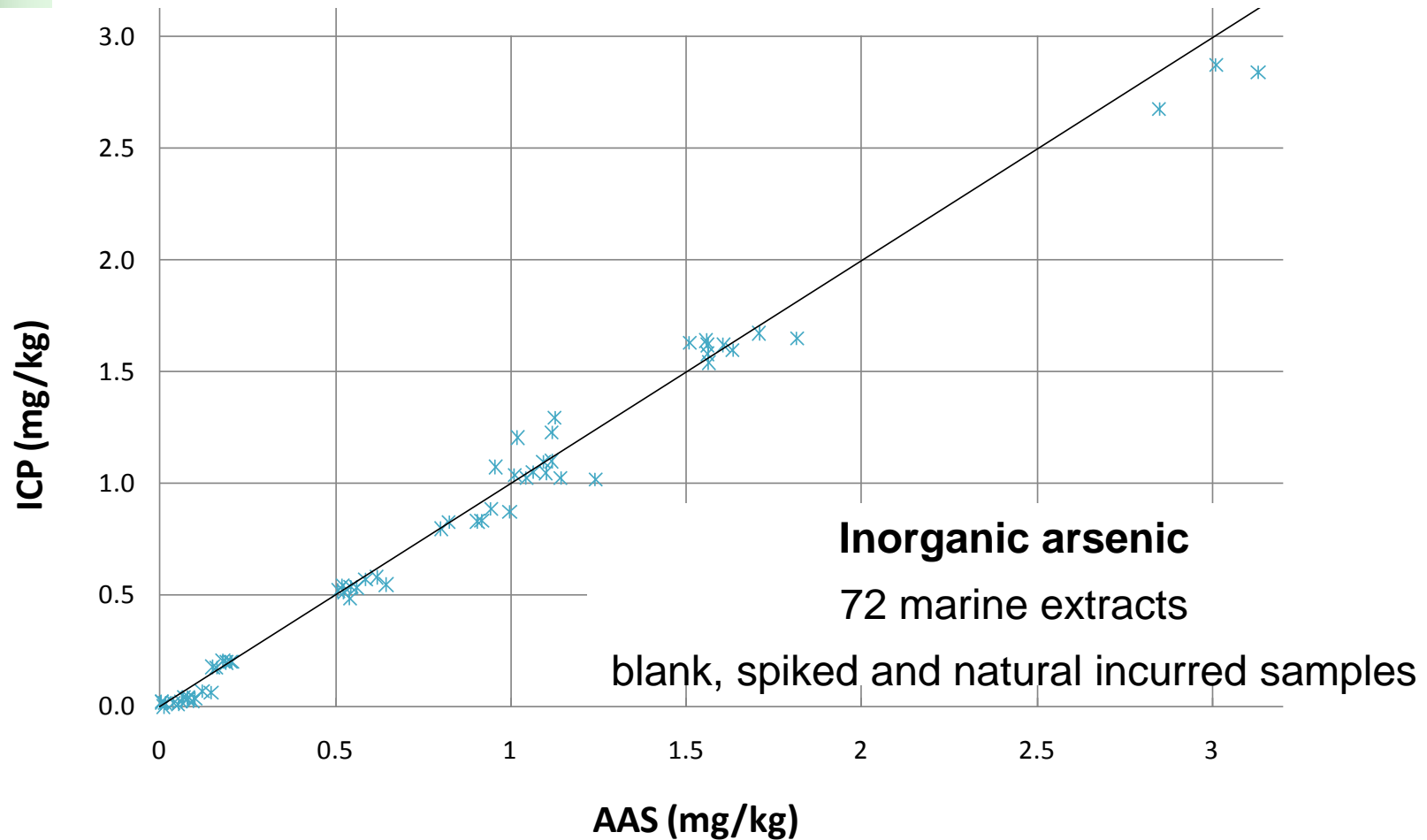
OrganoAs compounds

Inorganic As

Abundance



Inorganic arsenic: SPE-HG-AAS versus HPLC-ICP-MS



**The detection methods were not significantly different
(*t* Test, 95% confidence)**

In-house validation – iAs by SPE-HGAAS

Setup

- Spiked samples → Trout, oyster
- Natural incurred samples → TORT-2, DORM-3
- Analysed in triplicates on 3 different days
- 2 technicians

Results overview

- 0.08 mg/kg limit of detection (LOD)
- 3-8% repeatability
- 5-13% reproducibility
- 90-104% recovery

	Spike low	Spike medium	Spike high	TORT-2	DORM-3
iAs level (mg/kg)	0.5	1	1.5	0.9*	0.2*
Observations (N)	9	9	9	6	6
Mean recovery (%)	101	103	104	100	90
Repeatability RSD _r (%)	4	8	5	3	7
Reproducibility RSD _{IR} (%)	5	9	6	9	13
Horwitz Rel. Std. (%)	18	16	15	16	20

*Reference value determined by HPLC-ICP-MS



Collaborative trial – marine samples

Sample	Description	~conc level (mg/kg)
WP3-2	IMEP32-4 fish meal spiked	1
WP3-3	IMEP32-5 fish fillet spiked	2.5
WP3-4	Blue mussel powder	0.3
WP3-5	Crab powder	0.1
WP3-6	DORM-3 Dogfish muscle	0.2
WP3-7	TORT-2 Lobster Hepatopancreas	0.8

- 10 labs (one lab gave 2 sets of results => 11 datasets)
- SPE separation procedure was followed
- Both HG-AAS and ICPMS were used for determination of iAs



Collaborative trial – marine samples

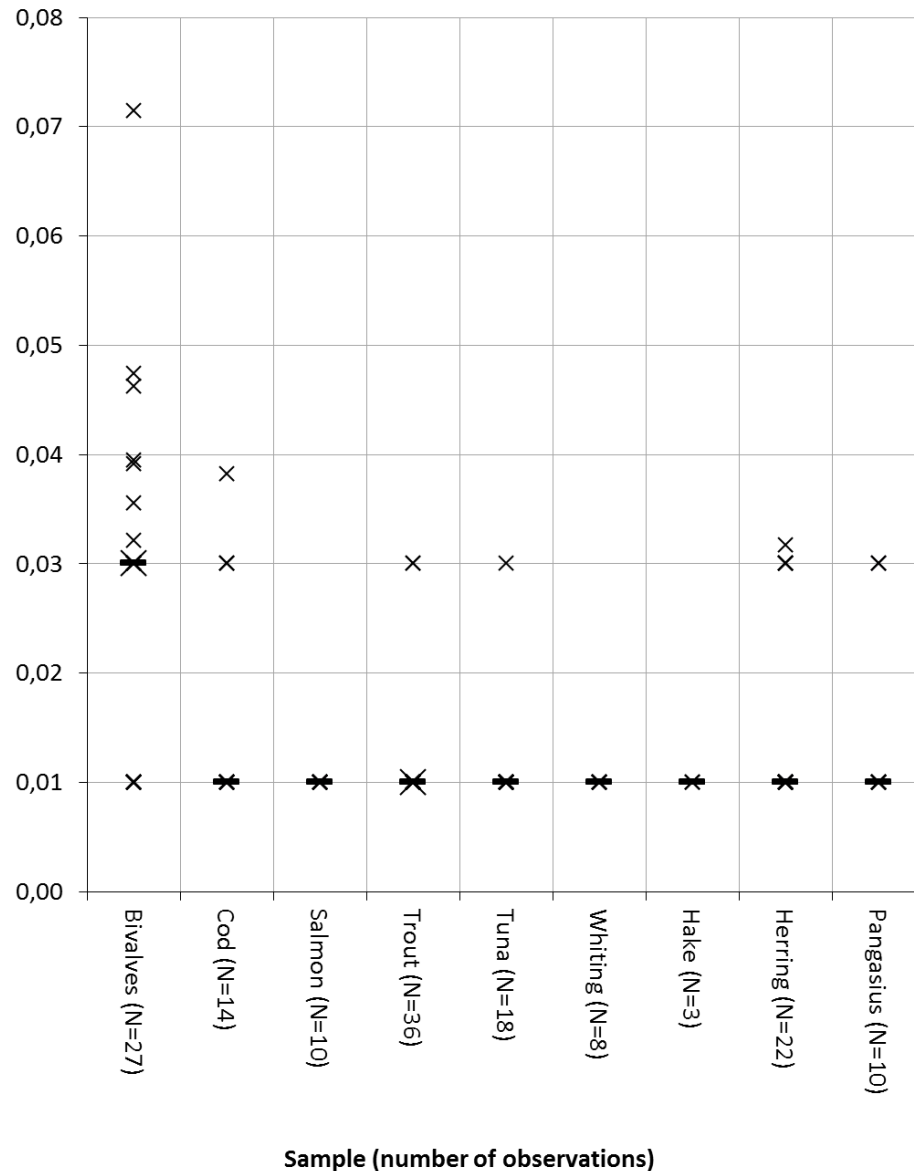
	Unit	WP3-2	WP3-3	WP3-4	WP3-5	WP3-6	WP3-7
No of labs		11	11	11	11	11	11
No of non-compliant labs		3	2	6	1	1	3
No of compliant labs		8	9	5	10	10	8
Overall mean	mg kg ⁻¹	1,03	2,57	0,26	0,14	0,19	0,76
S_r	mg kg ⁻¹	0,12	0,20	0,04	0,03	0,02	0,06
RSD _r	%	11,5	7,9	14,1	23,2	13,1	7,6
r_L	mg kg ⁻¹	0,33	0,57	0,10	0,09	0,07	0,16
S_R	mg kg ⁻¹	0,17	0,34	0,07	0,09	0,04	0,13
RSD _R	%	16,5	13,4	26,7	64,1	22,1	17,4
R_L	mg kg ⁻¹	0,47	0,96	0,19	0,26	0,12	0,37
Horwitz value		15,8	13,8	19,5	21,3	20,4	16,6
HorRat		1,0	1,0	1,4	3,0	1,1	1,1

Low conc

- Precision: RSD_r : 8 - 14% and RSD_R :13 - 27%
- Accuracy: 89-100%
- Measurement range: 0.2 - 2.6 mg/kg
- HorRat: 1.0 – 1.4
- HG-AAS vs ICPMS: no difference
- Blue mussel sample (WP3-4): not satisfactory results



Survey data – marine samples



Inorganic arsenic

- 148 seafood samples
- all fish <0.04 mg/kg
- bivalves <0.01 – 0.07 mg/kg



Inorganic arsenic in wild caught fish => no concern



Norwegian survey

900 individual fish samples

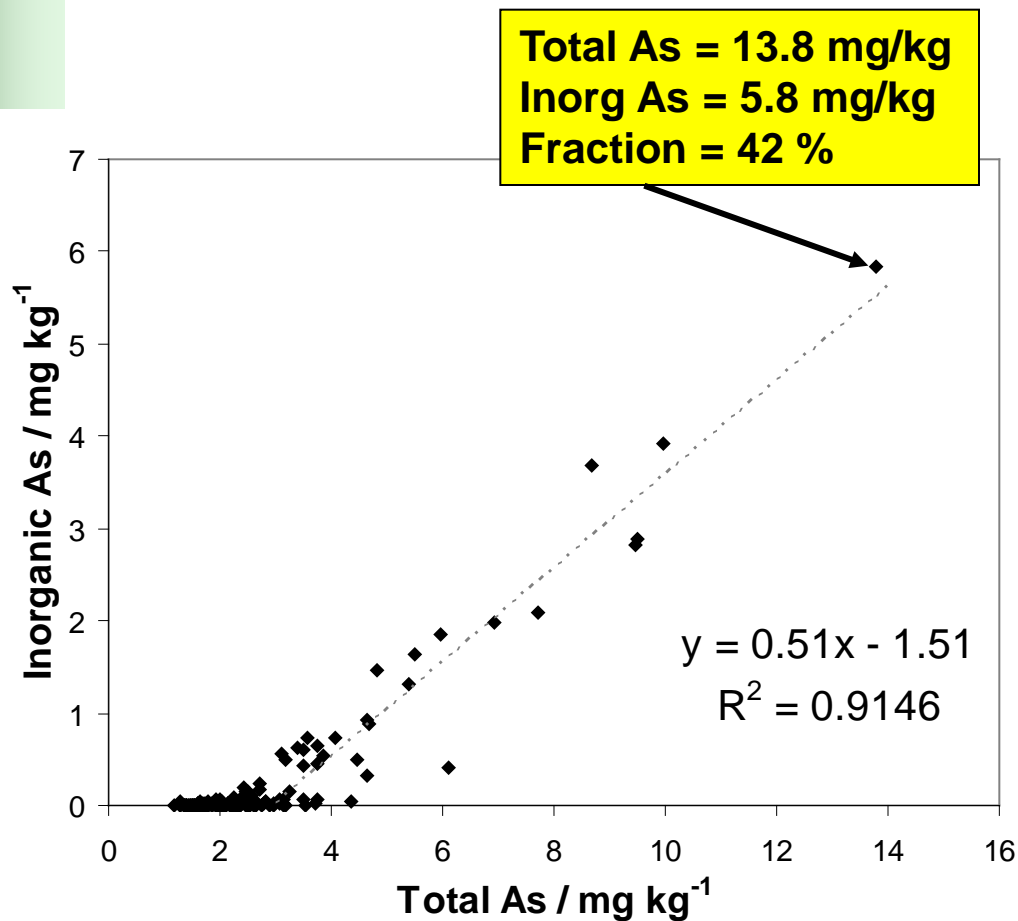
- Atlantic halibut
- Cod
- Greenland halibut
- Mackerel
- Herring
- Tusk

Results

Total arsenic.....0.3-110 mg/kg

Inorganic arsenic.... < 0.01 mg/kg
(only 37 samples > LOQ)

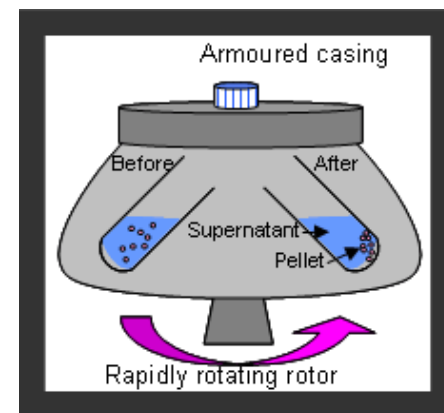
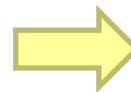
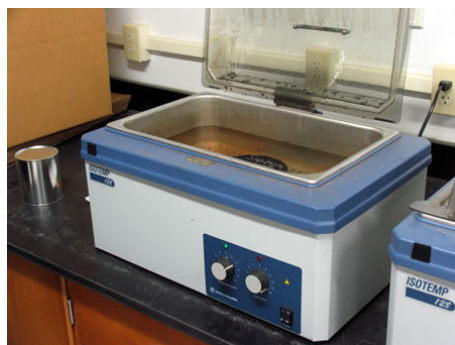
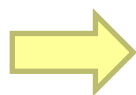
...but in bivalves high contents in some samples...



Data from 175 blue mussel (*Mytilus edulis*) samples collected along the Norwegian Coastline.



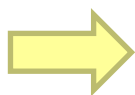
SPE HG-AAS – iAs in rice



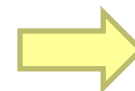
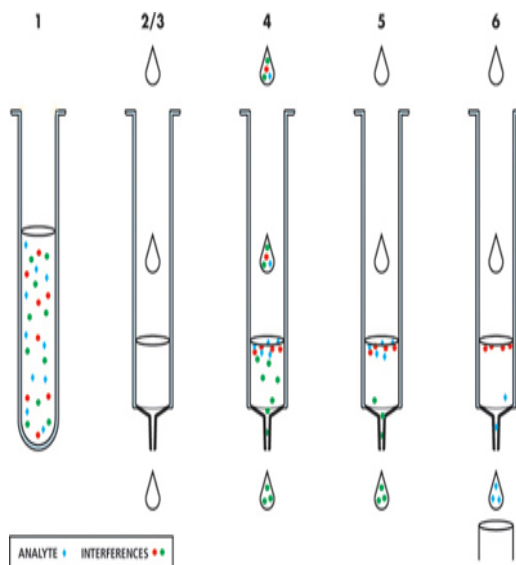
Sample + 10 mL extractant
(0,1 M HNO₃, 3% H₂O₂)

90°C waterbath, 1h

centrifugation



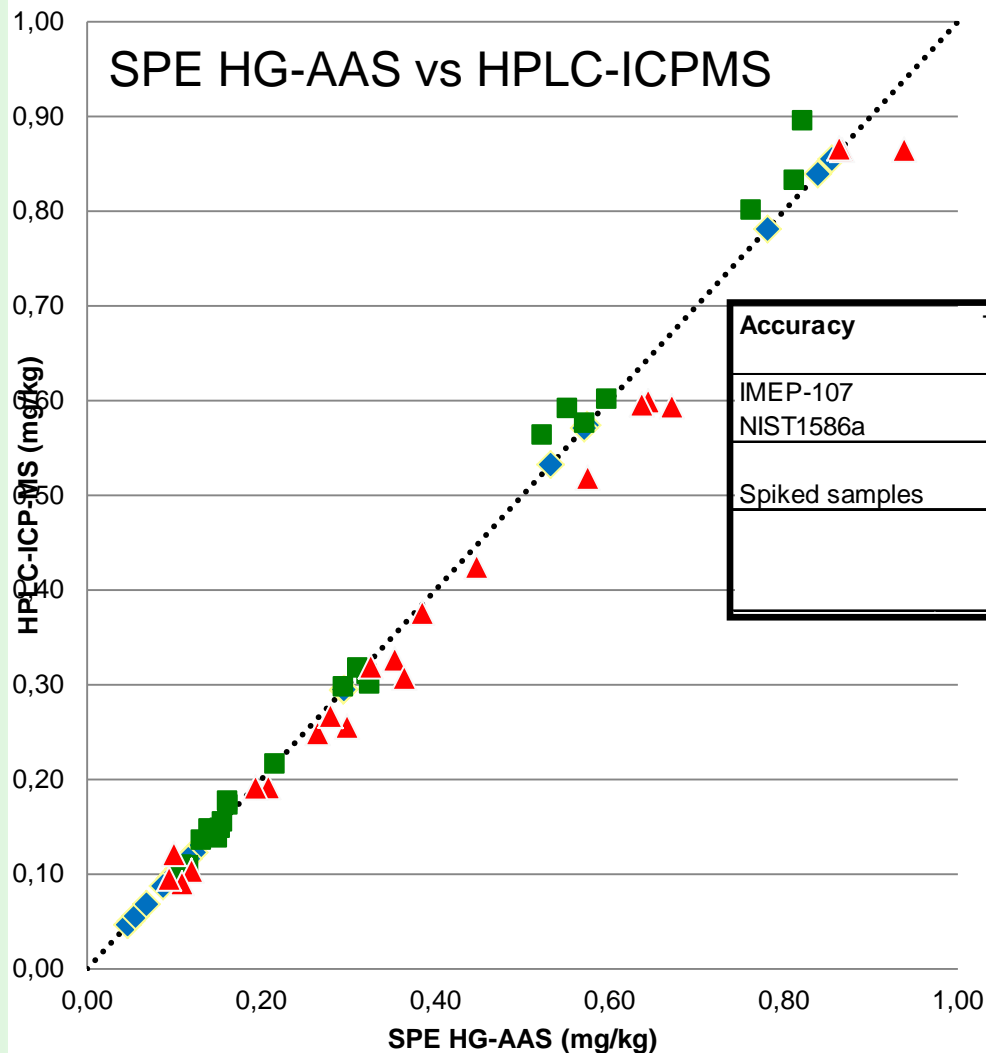
SPE separation



HG-AAS



SPE HG-AAS – iAs in rice - validation



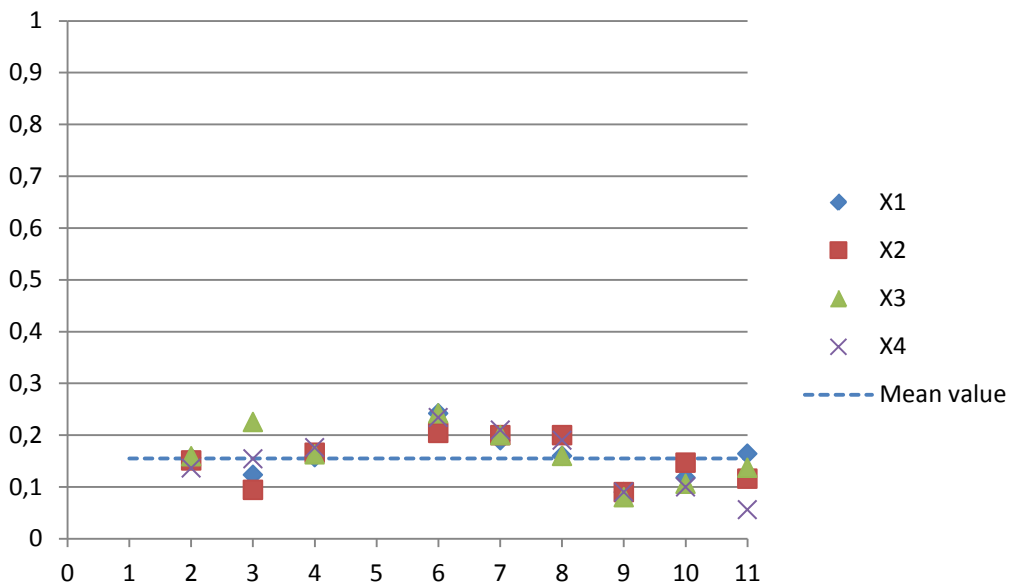
Accuracy	Target value (mg/kg)	Found (mg/kg) mean +/- 2s	
IMEP-107	0,107 +/- 0,014	0,108 +/- 0,017	(N=6)
NIST1586a	0,097	0,101 +/- 0,014	(N=6)
Spiked samples	Spike level	Recovery	
	0,30 mg/kg	105 %	(N=9)
	0,55 mg/kg	106 %	(N=9)
	0,80 mg/kg	106 %	(N=9)

Precision		
Repeatability	RSDr	4,8 %
Reproducibility	RSDR	7,8 %

LoD / LoQ		
LoD	(k=3)	0,02 mg/kg
LoQ	(k=6)	0,04 mg/kg



SPE HG-AAS – iAs in rice – collaborative study



WP3-9	
No of labs	11
No of non-compliant labs	2
No of compliant labs	9
Overall mean	0,16
S_r	0,03
RSD_r	18,3
r_L	0,08
S_R	0,05
RSD_R	30,0
R_L	0,13
Horwitz value	21
HorRat	1,4



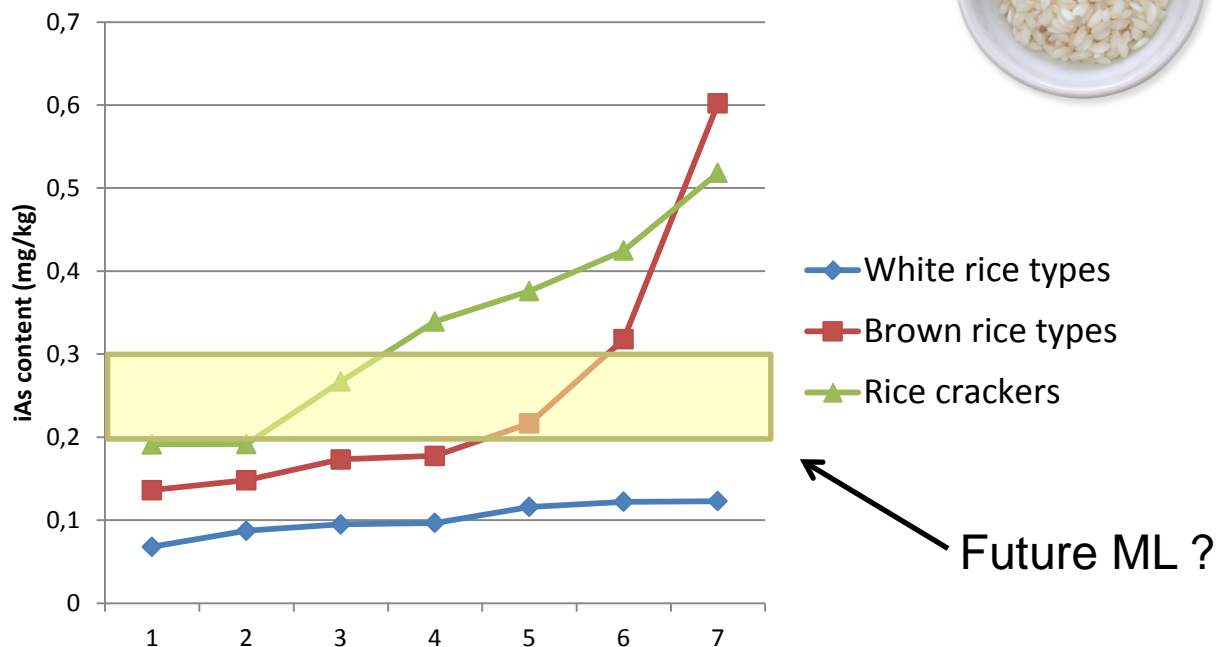
Test sample:
Wholemeal rice flour
(organic)



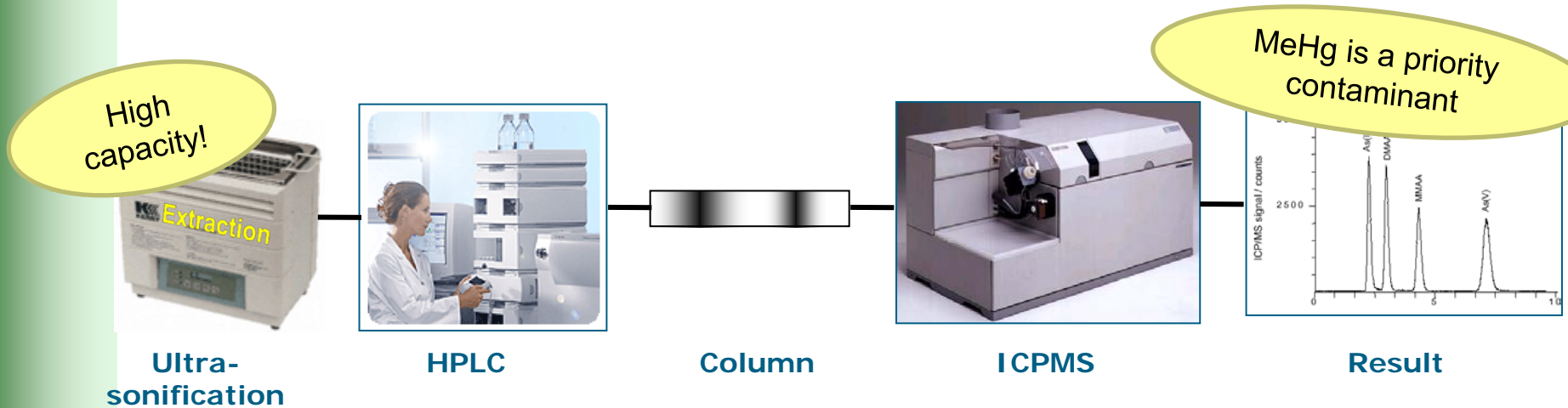
Survey data – iAs in rice samples

21 samples (so far)

- White rice
- Brown rice types
- Rice crackers

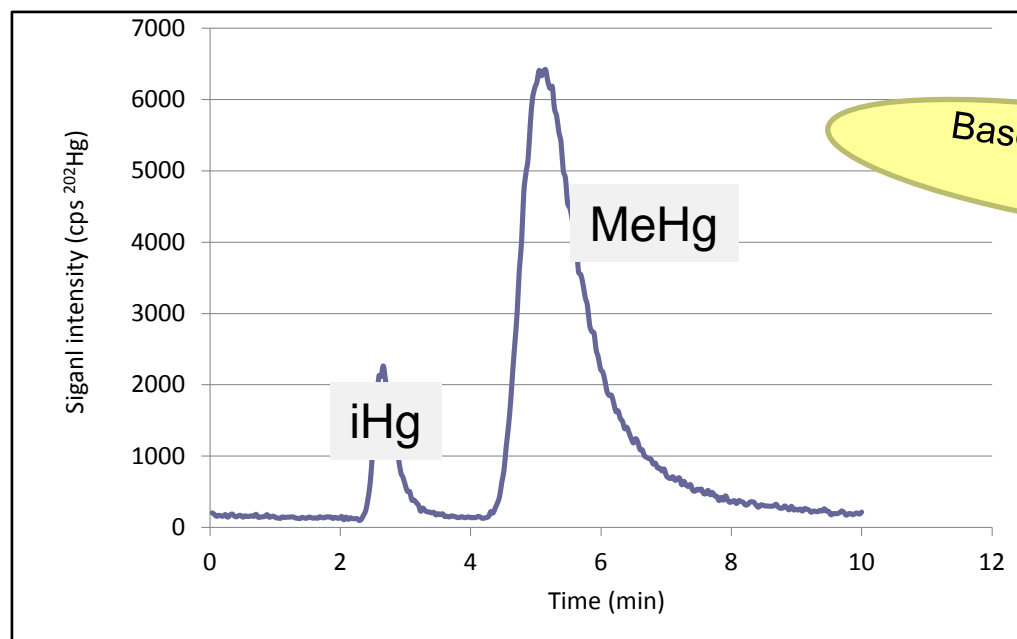


Speciation analysis of mercury by HPLC-ICPMS



- 0.5 gram sample (2 x extraction with 5 ml 5 M HCl)
- Centrifugation
- pH adjustment
- Cation exchange (Hamilton PRP X200 SCX)
- HPLC-ICPMS

Cation exchange HPLC-ICPMS



HPLC-ICPMS chromatogram of DORM-3 (Dogfish muscle)



Performance of the HPLC-ICP-MS method for determination of MeHg

	DORM-2 <i>Dogfish</i>	TORT-2 <i>Lobster</i>	DORM-3 <i>Dogfish</i>	Fishfeed #1	Fishfeed#2	Codfish	Salmon
Ref level (mg/kg)	4.47	0.15	0.36	0.21	0.06	0.17	0.06
Observations (N)	9	15	9	9	9	9	9
Mean recovery (%)	94	102	96	-	-	-	-
Repeatability RSD _r (%)	3	4	3	11	13	5	13
Reproducibility RSD _{IR} (%)	8	12	8	11	15	12	20
Horwitz Rel. Std. (%)	13	21	19	20	25	21	25

Setup

- Natural incurred samples
 - CRMs (DORM-2, DORM-3 and TORT-2)
 - fish feed, codfish and salmon
- Analysed in triplicates on 3 different days
- 2 technicians

Results overview

- 0.004 mg/kg limit of detection (LOD)
- Mean repeatability = 7%
- Reproducibility < Horwitz RSD
- 94-102% recovery



Collaborative trial – marine samples

- Small scale ILC (4 labs)
- 6 samples (0,15 – 5,5 mg/kg)
- Both seafood and feed

	Target value	LAB1	LAB2	LAB3	LAB4
WP3-1 Complete feed (spiked)	0,19	0,21	0,20	Data to be produced Sept/Oct	
WP3-3 Fish fillet (spiked)	1,8	2,08	1,91		
WP3-5 Crab powder	0,28	0,35	0,34		
WP3-6 DORM-3	0,355	0,38	0,34		
WP3-7 TORT-2	0,152	0,17	0,15		
WP3-8 CE464 Tunafish	5,5	5,53	5,61		



Survey data - MeHg in fish feed and ingredients

Type	Sample ID	% Fat	Hg (total) (µg/kg)	MeHg (µg/kg)
Fish silage	204557	11.8	39	<30
	205398	11.3	40	<30
	207967	10.7	39	<30
	207976	9.2	11	<30
	208547	11.3	55	<30
Fish oil	201224	100	<10	na
	201225	100	<10	na
	205376	100	<10	na
Complete feed	207847	34.6	24	<30
	210554	28.8	18	<30
	210555	17.0	36	<30
	210606	24.8	49	32
Fish meal	201226	13.7	120	125
	201227	14.0	93	79
	202128	13.7	71	45
	202141	8.2	48	30
	204687	12.0	30	<30
	204836	10.3	43	<30
	206945	10.4	34	<30
	207833	12.0	33	<30
	207899	12.3	27	<30
	210705	11.0	69	53
	211035	6.0	67	55
	211612	7.9	40	<30
	211662	14.4	61	53
	211669	9.7	44	32

All samples collected as part of the national surveillance/feed-control programme in Denmark

EU maximum level

- No ML for MeHg
- 0.2 mg/kg for total Hg (2010) (before 2010 the ML= 0.1 mg/kg)
- all samples < ML



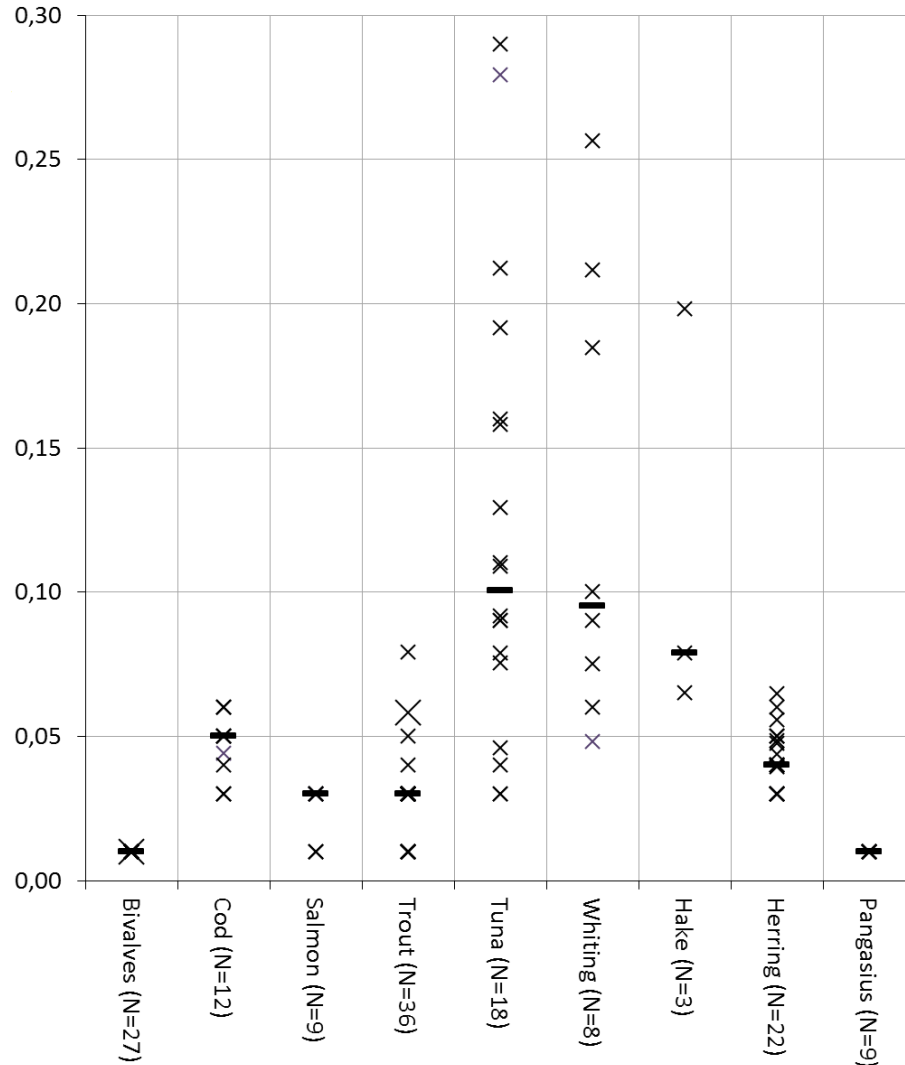
Survey data – MeHg in seafood

Methyl mercury in fish and fish feed

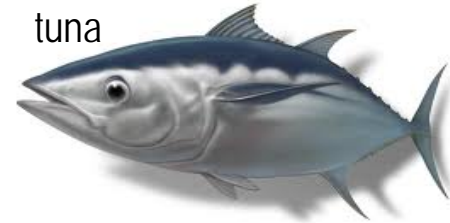
mg/kg

LOD = 0.01 mg/kg

LOQ = 0.03 mg/kg



tuna



Whiting



Hake



Herring



Cod



salmon



pangasius



bivalves



Output from CONffIDENCE WP3

Methods:

- iAs in marine samples by SPE HG-AAS
- iAs in rice samples by SPE HG-AAS
- MeHg in marine samples by HPLC-ICPMS

Collaborative trials:

- iAs in marine samples by SPE HG-AAS (10 labs)
- MeHg in marine samples by HPLC-ICPMS (4 labs)
- "target values" established for future QA purposes

Survey data:

- iAs in marine samples (N=130)
- iAs in rice samples (N=30)
- MeHg in marine samples (N=130)

Contribution to risk-benefit analysis :

- Seafood samples analysed for POPs and fatty acids (with WP1)
- Reported to EFSA databases for future risk evaluations



Further information

- www.confidence.eu
- CONffIDENCE newsletters
- Scientific publications
 - Hedegaard and Sloth, Heavy metal speciation in feed: why and how?, *BASE*, 2011, 15, 45-51.
 - Rasmussen *et al*, Development and validation of an SPE HG-AAS method for determination of inorganic arsenic in samples of marine origin, *Anal Bioanal Chem*, 2012, 403, 2825-2834.
 - Rasmussen *et al*, Development and validation of a HPLC-ICPMS method for determination of methylmercury in marine food and feed, *Anal Bioanal Chem* (CONffIDENCE special issue), *in prep* (expected 2013)
 - Sloth *et al*, Contaminant and fatty acid profiles in European seafood, *in prep* (expected 2013)
- Contact: Jens J. Sloth (jjsl@food.dtu.dk) (WP3 leader)

➤ Thanks for your attention!

