Work package 3

DTU Food
National Food Institute

WP leader
Jens J. Sloth

Heavy metals

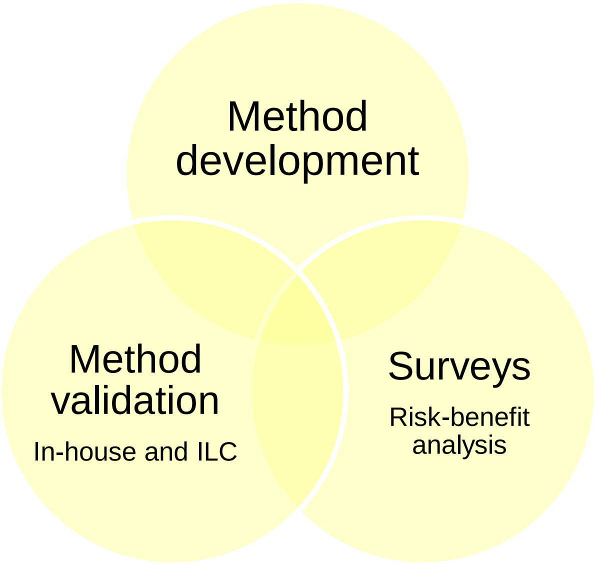
CONFfIDENCE
Stakeholder workshop
Brussels 20. September 2012

SEVENTH FRAMEWORK PROGRAMME

www.conffidence.eu

Agenda

- **The CONffIDENCE project**
- **WP3 on ”heavy metals”**
- **Inorganic arsenic**
  - SPE HG-AAS method
  - seafood samples
  - rice samples
- **Methylmercury**
  - HPLC-ICPMS method
  - seafood samples
  - feed samples



CONffIDENCE in a nutshell

CONtaminants in **F**ood and **F**eed –

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, dithiocabamates	
➢	Veterinary drugs:	- antibiotics, e.g. tetracyclines - coccidiostats, e.g. ionophores
➢	**Heavy metals speciation:**	**-inorganic arsenic** **-methylmercury**
➢	Biotoxins:	- alkaloids - marine biotoxins - mycotoxins

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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 µg/kg bw per day for inorganic arsenic
- => EU dietary exposures within this range
- => Risk to some consumers cannot be excluded

- **NEW!** $BMDL_{0.5}$ = **3 µg/kg bw per day** for inorganic arsenic
  => 0.5% increased incidence of lung cancer for 12 y exposure

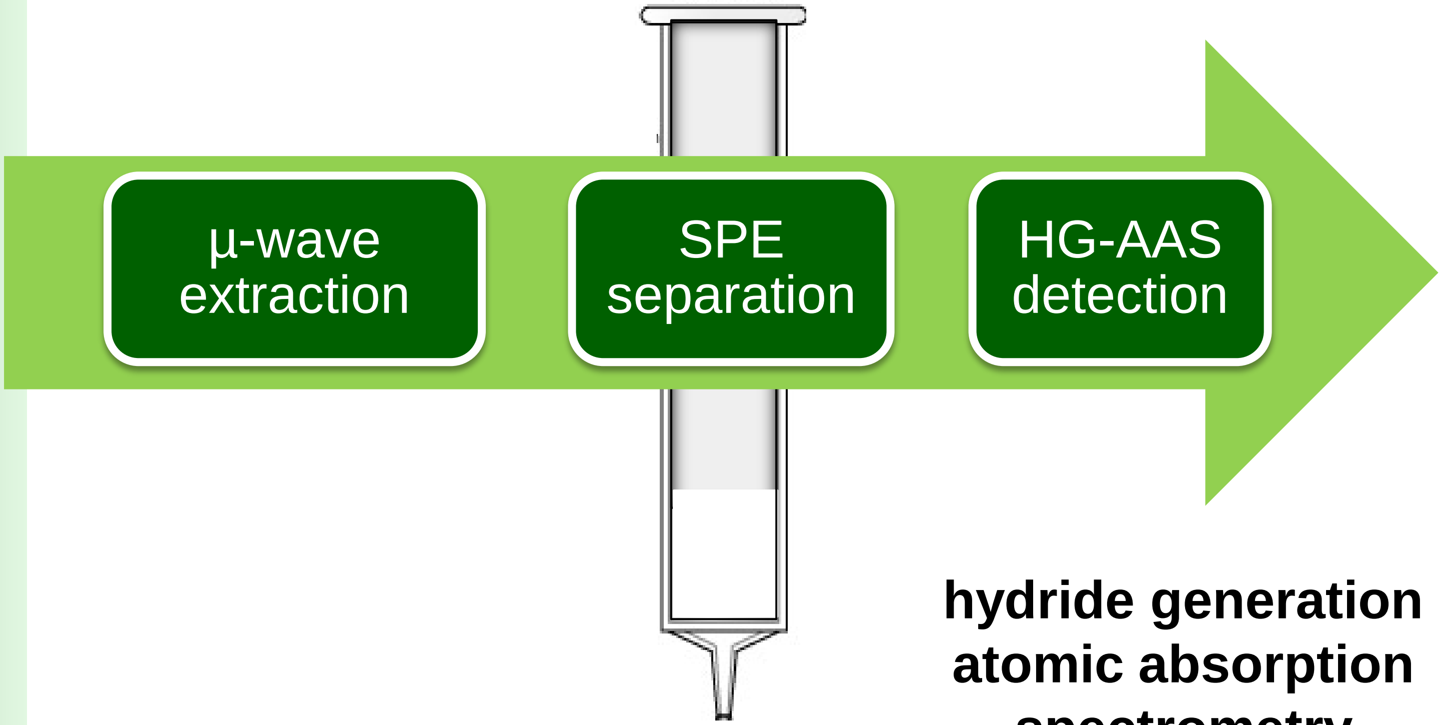
- *"…there is a need to produce speciation data for different food commodities to support dietary exposure assessment…"*

- *"…more accurate information on the inorganic arsenic content of foods is needed to improve assessments of dietary exposures to inorganic arsenic"*

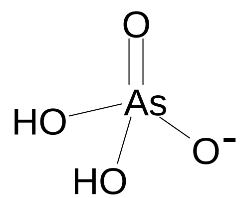
- *"…need for validated methods for selective determination of inorganic arsenic in food matrices"*

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)

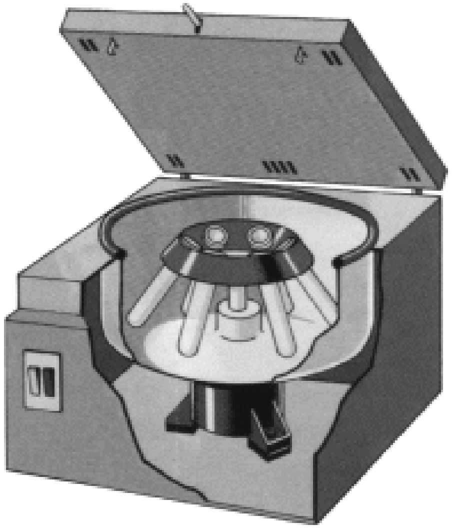
0.2 g sample
+ 10 mL extractant
(0.06 M HCl, 3% H_2O_2)

Glas vessel

25 minutes at 90°C

µ-wave oven

Centrifugation
10 min 2100 x *g*



centrifuge

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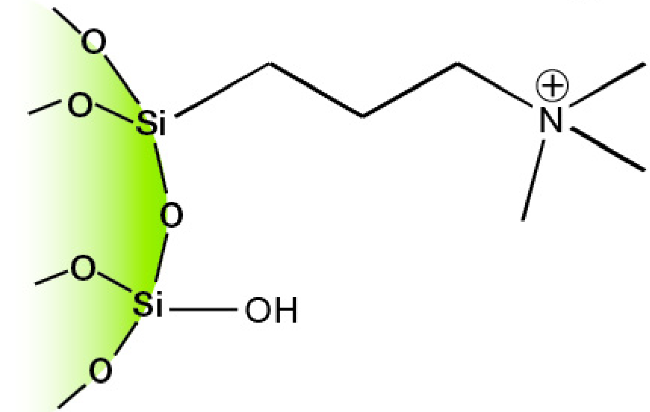
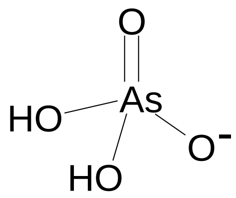
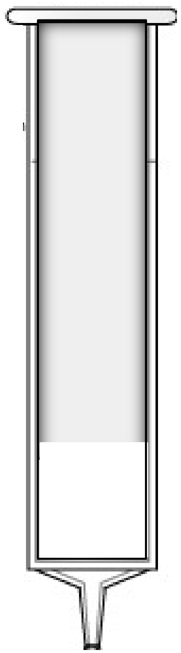
SPE protocol - Separation of As species





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

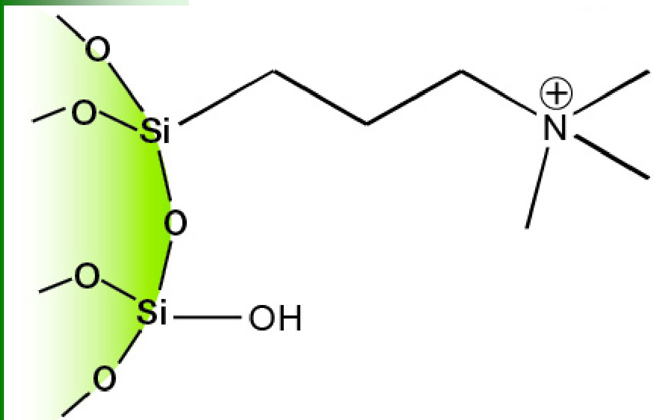


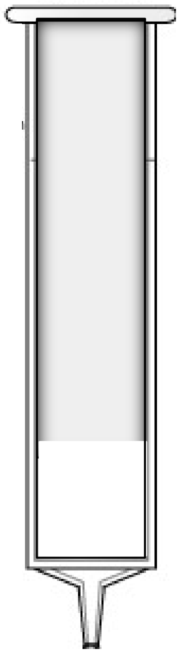
The **charge** of the arsenic species depends on pH

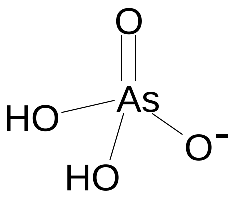
@ pH = 6 **iAs(V)** is **negatively charged**

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

SPE protocol - Separation of As species







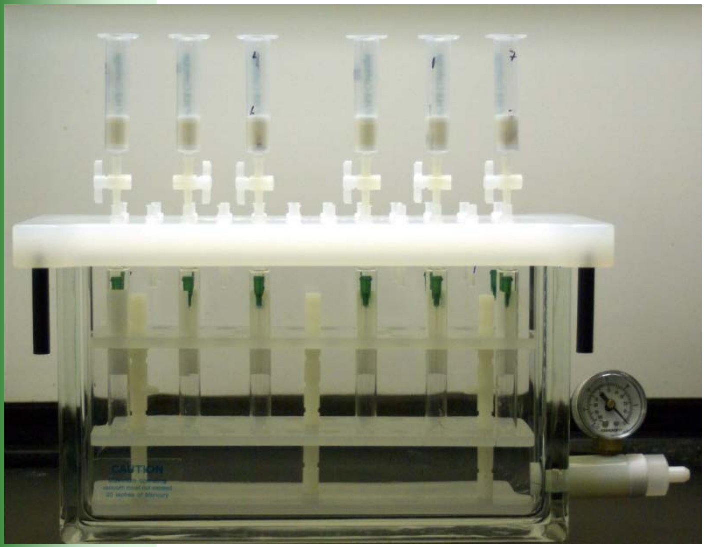
Condition
100 % MeOH

Equilibrate
Buffer: 20mM $(NH4)_2CO_3$, 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

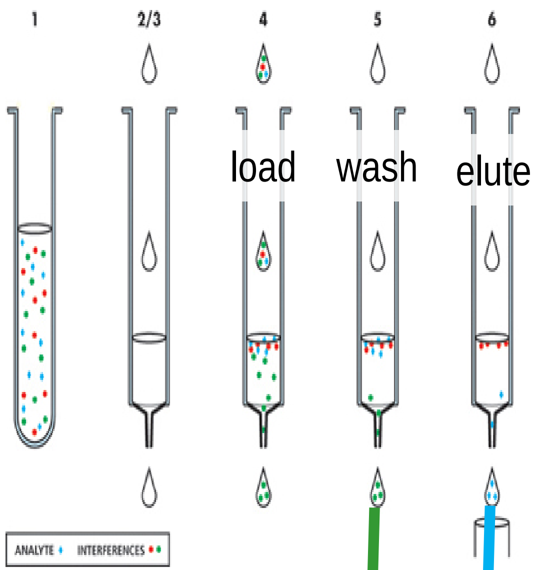


Illustrations: Crawfordscientific, Phenomenex and Jeff Dahl.

SPE-HG-AAS – a novel speciation alternative…

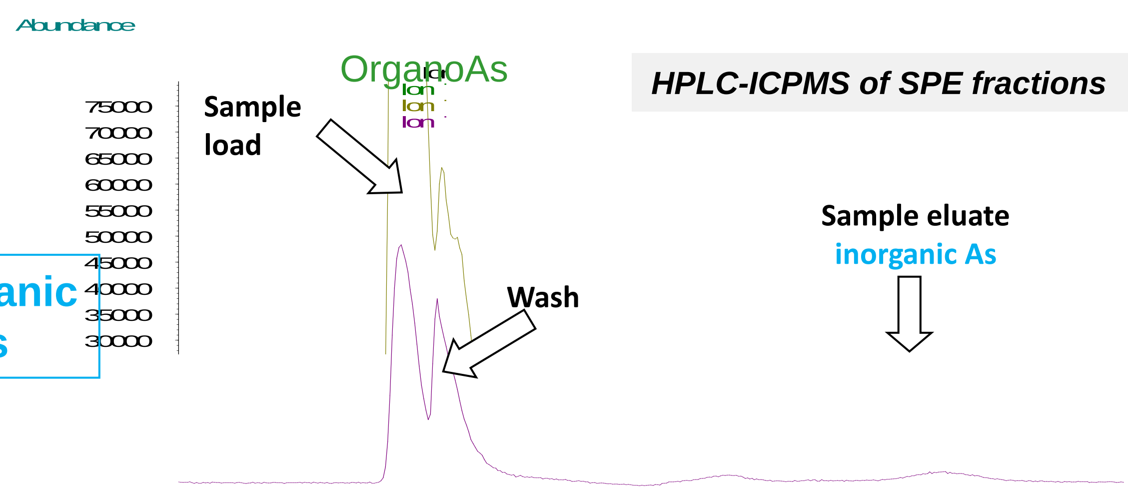
µ-wave extraction → Separation by SPE → Detection by HG-AAS

Inexpensive detection system

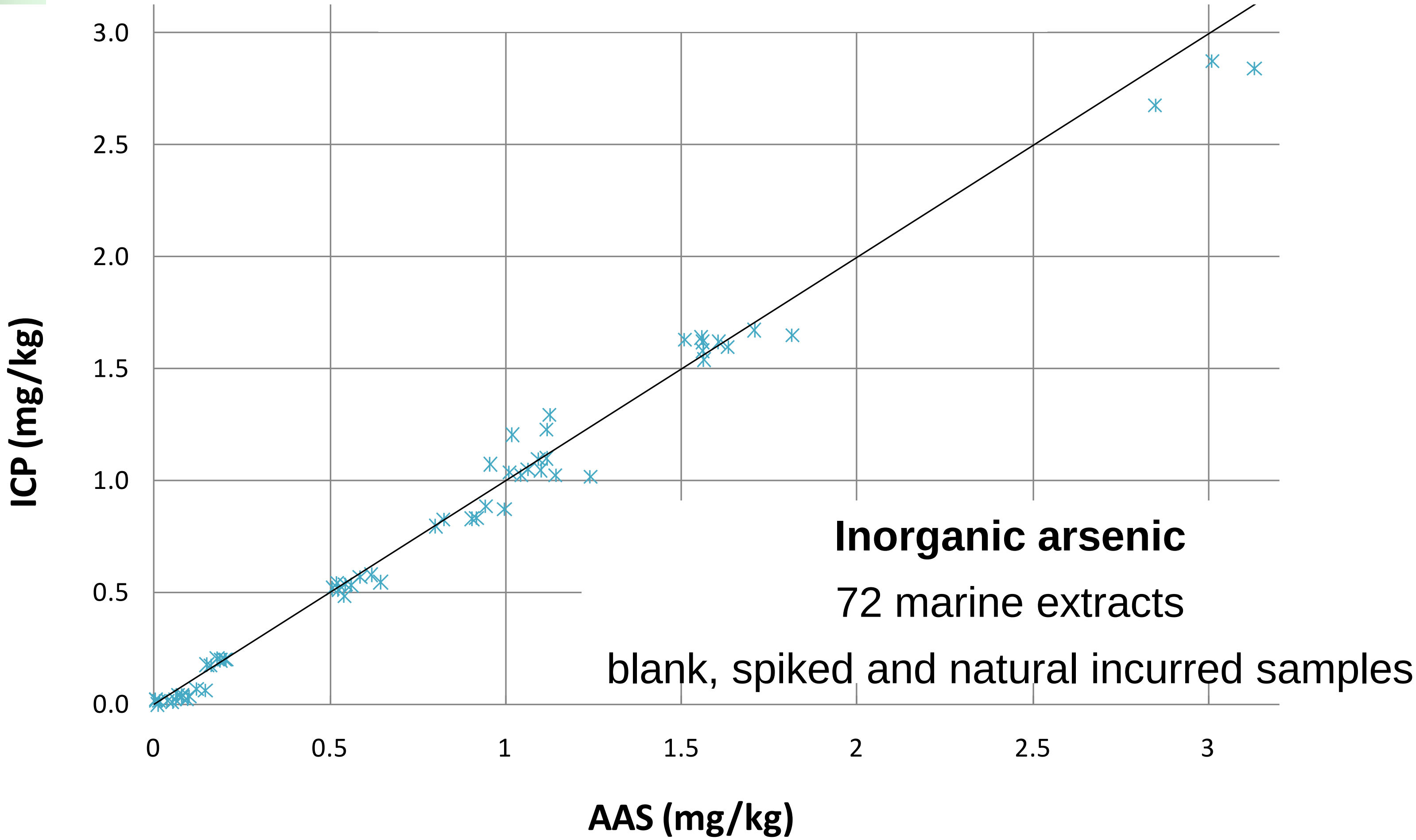


OrganoAs compounds | **Inorganic As**

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



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*

Rasmussen et al, ABC 2012

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 resutls)
- Both HG-AAS and ICPMS were used for determination of iAs

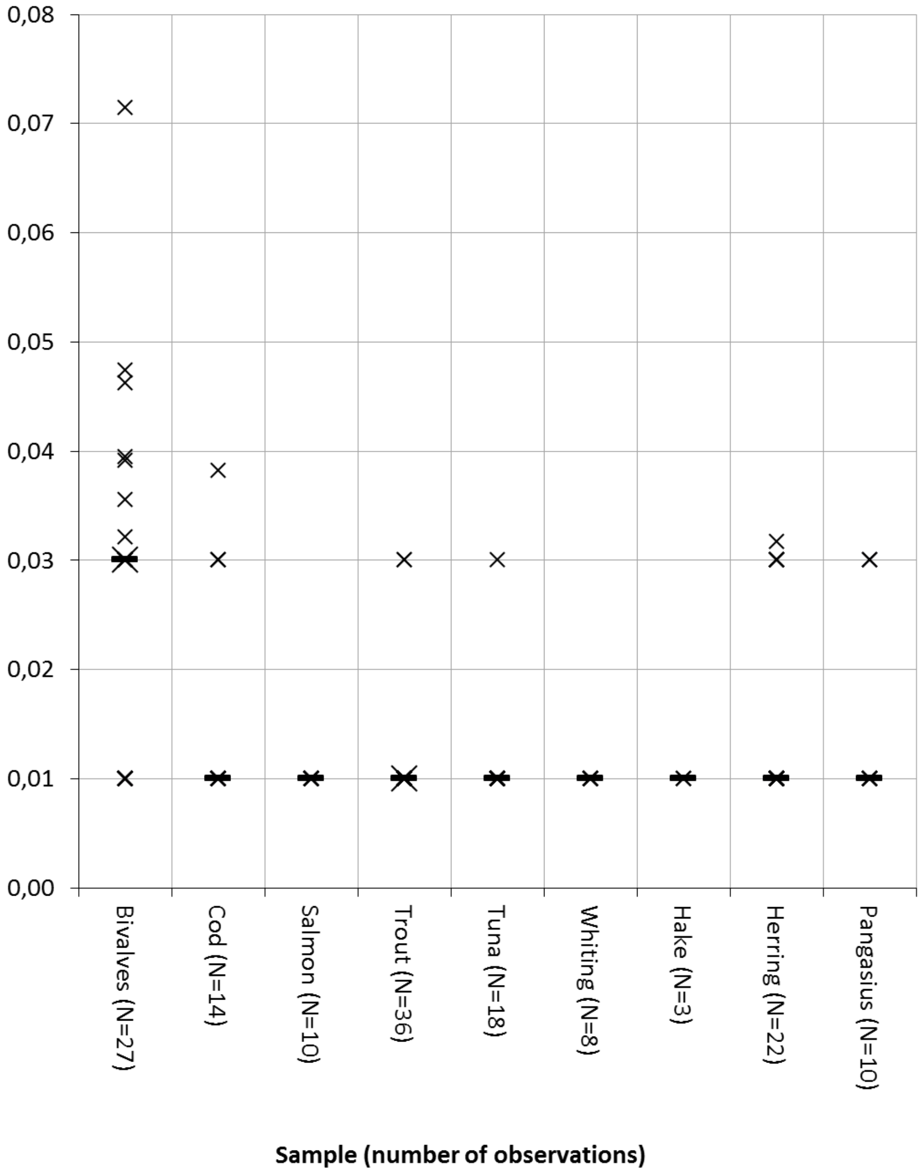
Collaborative trial – marine samples

	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

- Precision: RSD_r : 7.6 - 18.3% and RSD_R :13.4 - 30%
- 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

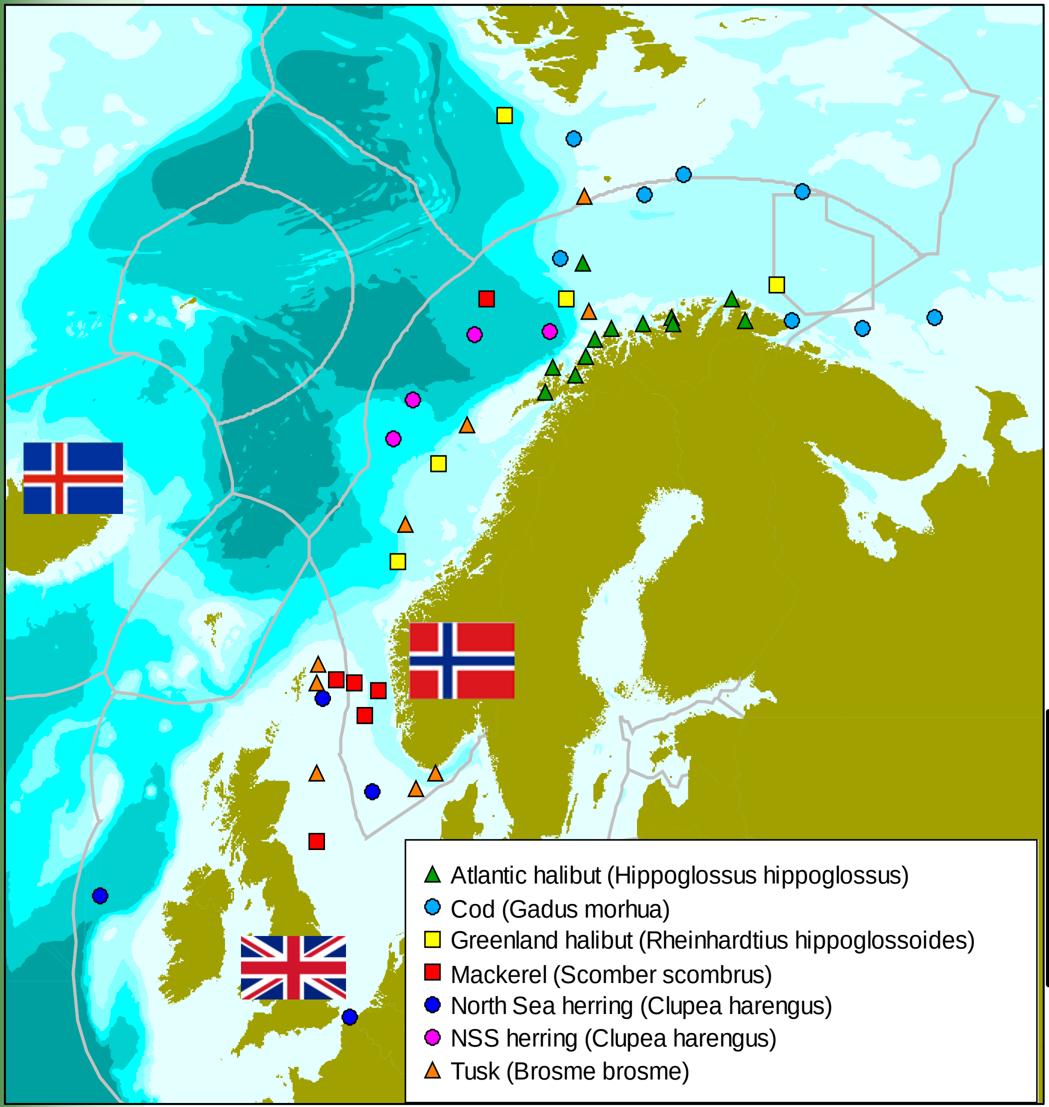
Survey data – marine samples



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

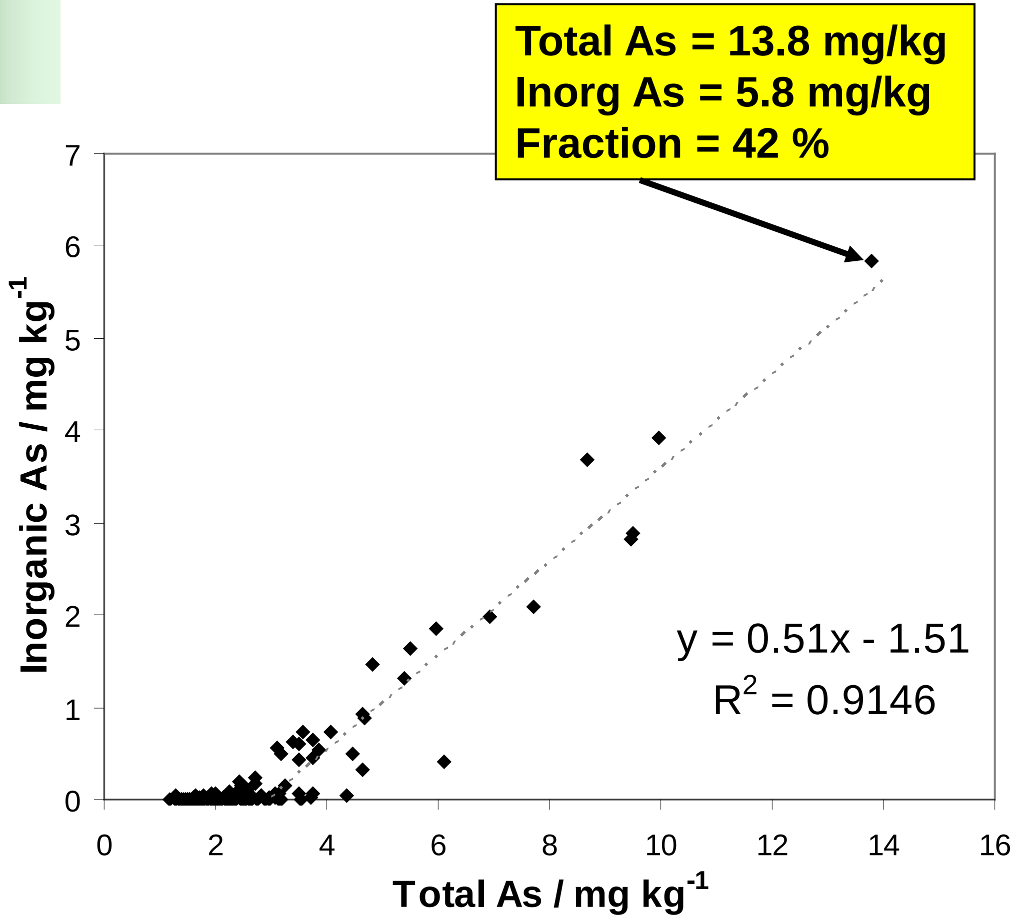
Inorganic arsenic…. < 0.01 mg/kg

(only 37 samples > LOQ)

NIFES NATIONAL INSTITUTE OF NUTRITION AND SEAFOOD RESEARCH

Julshamn and Sloth, Fd Addit Contam B, 2012, in press

…but in bivalves high contents in some samples…



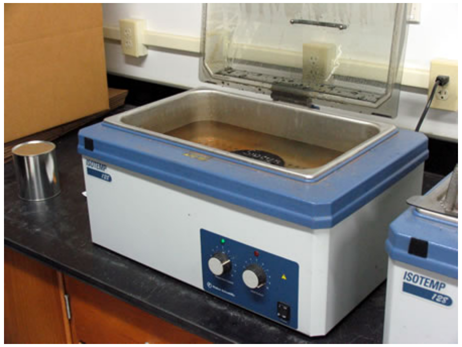
Data from 175 blue mussel (*Mytilus edulis*) samples collected along the Norwegian Coastline.

NIFES
NATIONAL INSTITUTE OF NUTRITION AND SEAFOOD RESEARCH

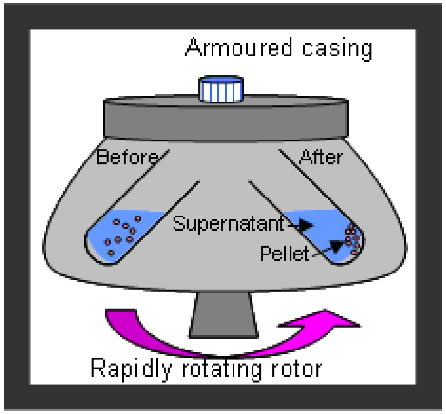
Sloth and Julshamn, 2008, J. Agri.Food Chem., 56, 1269-1273

SPE HG-AAS – iAs in rice

Sample + 10 mL extractant
(0,1 M HNO_3, 3% H_2O_2)

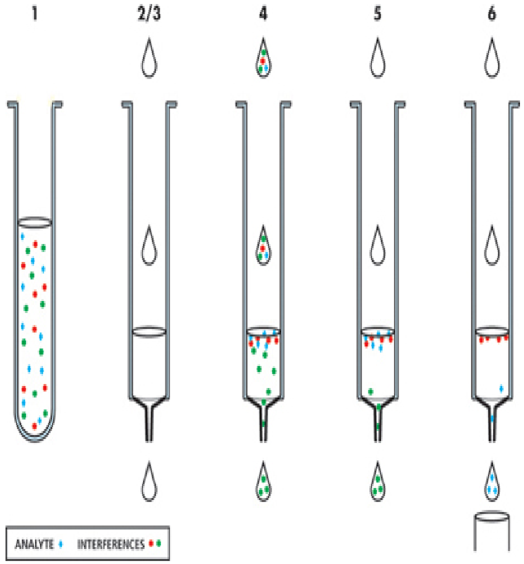


90°C waterbath,1h



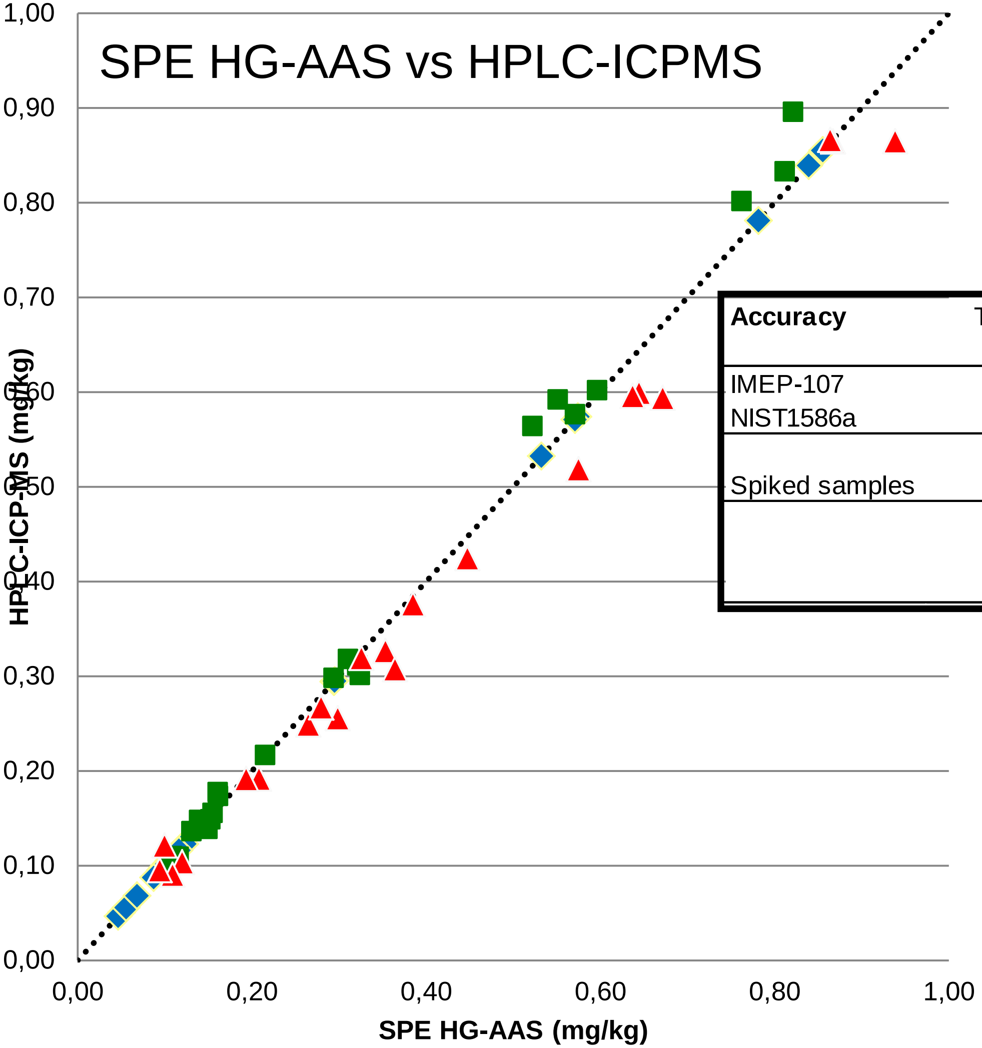
centrifugation

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)

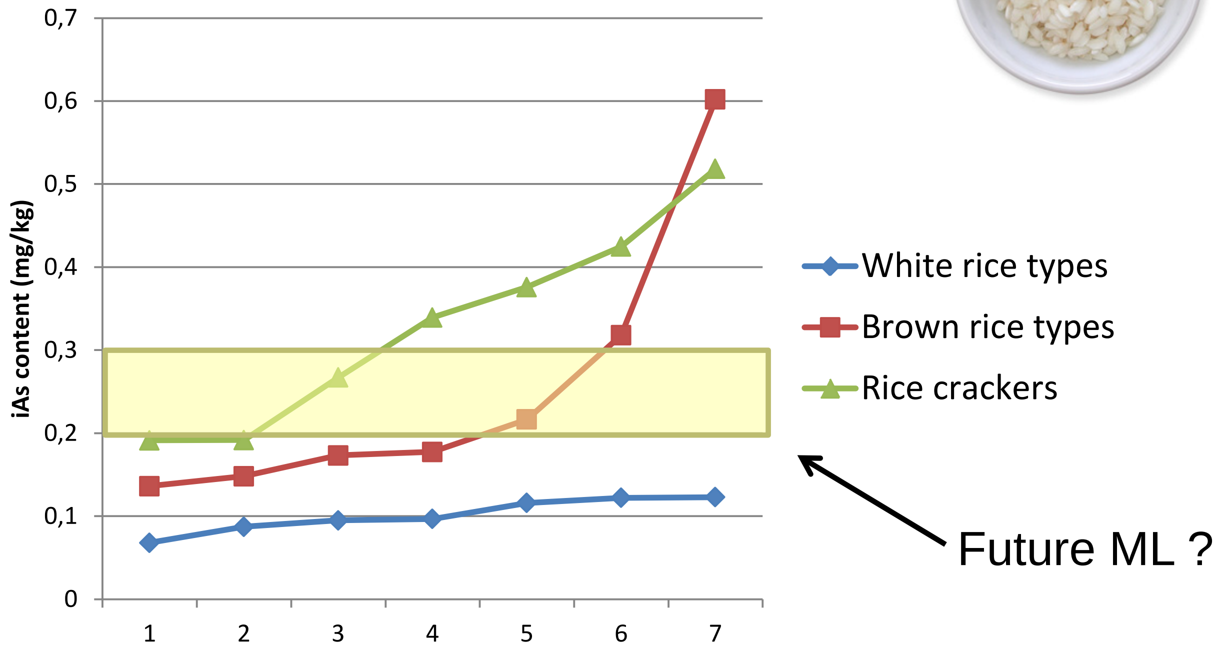
Precision		
Repeatability	RSDr	4,8 %
Reproducibility	RSDR	7,8 %

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

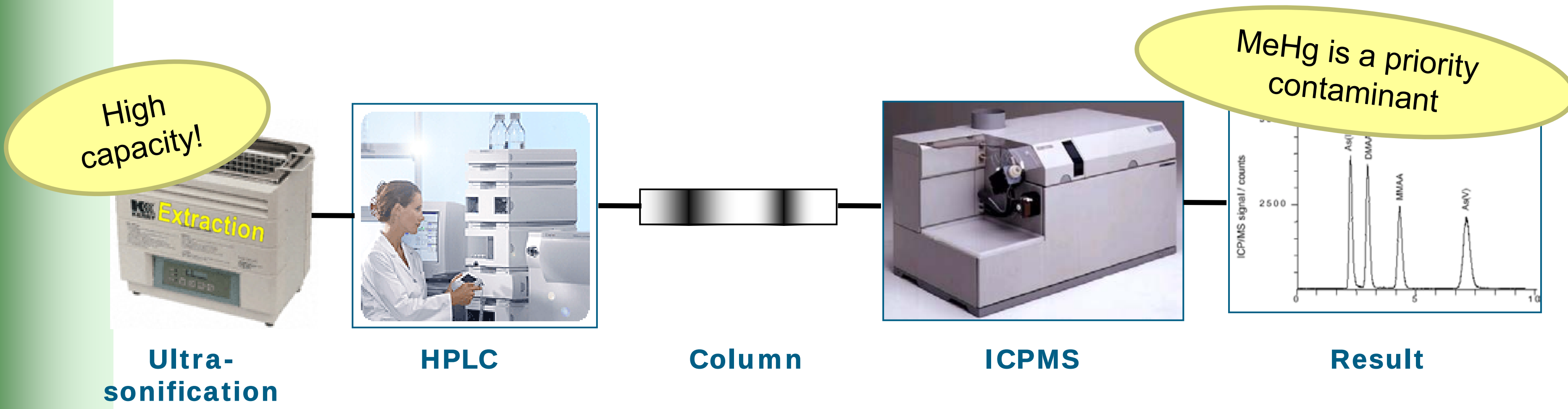
Survey data – iAs in rice samples

21 samples (so far)

- White rice
- Brown rice types
- Rice crackers

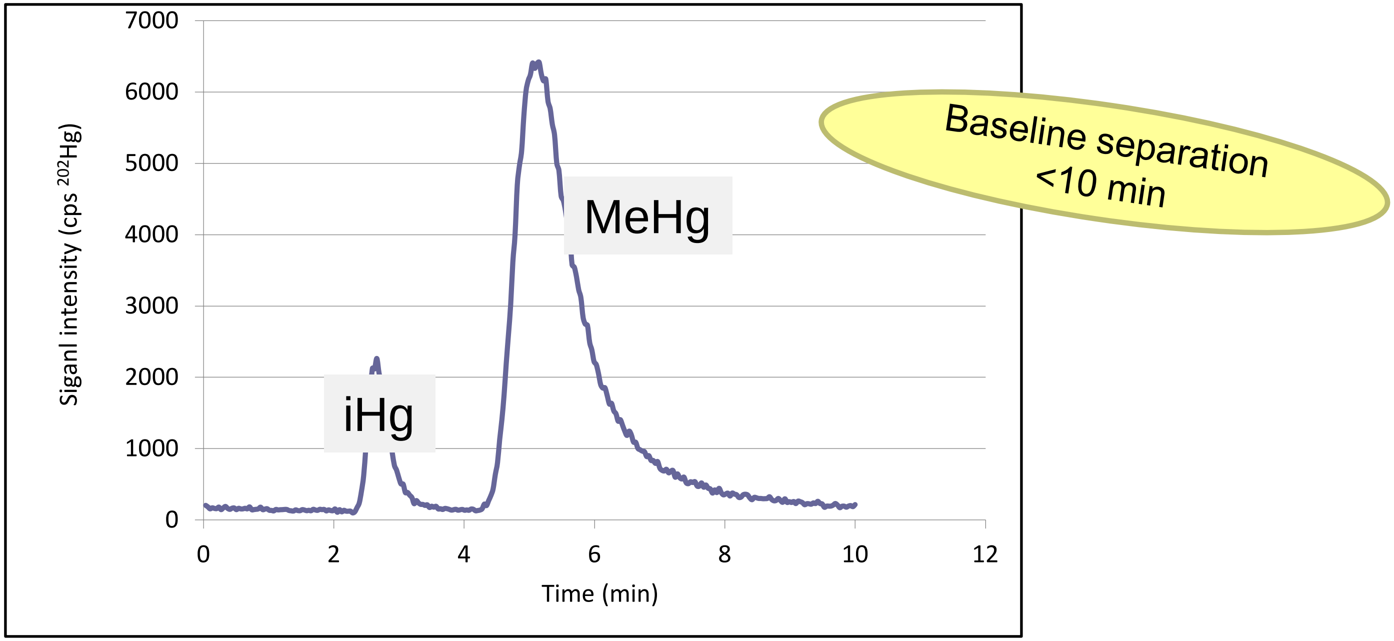


Speciation analysis of mercury by HPLC-ICPMS



- 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 methylmercury

	DORM-2 *Dogfish*	TORT-2 *Lobster*	DORM-3 *Dogfish*	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-2,3 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		
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		

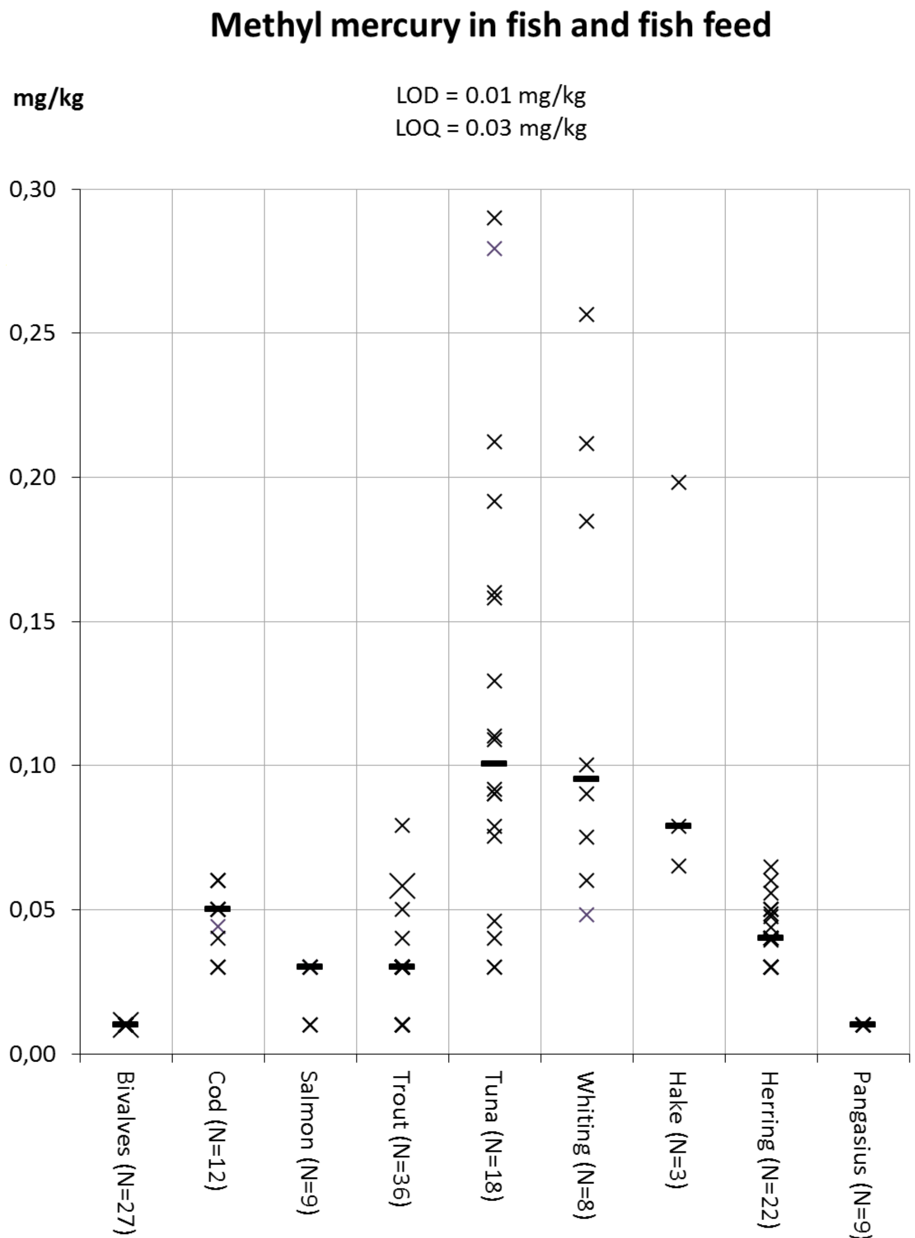
Survey data - MeHg in fish feed and ingredients

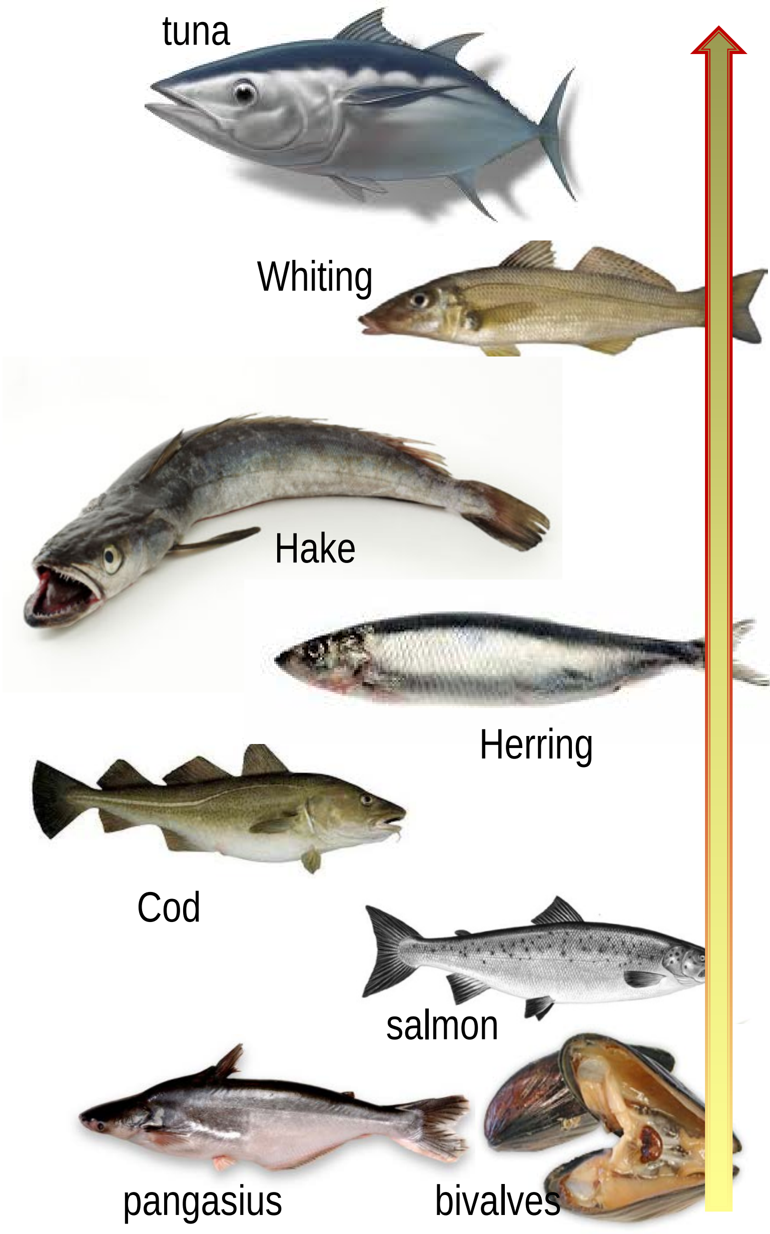
Type	Sample ID	% Fat	Hg (total) (µg/kg)	MeHg (µg/kg)
Fish silage	204557	11.8	39	<30
	205398	11.3	40	<30
	207967	10.7	39	<30
	207976	9.2	11	<30
	208547	11.3	55	<30
Fish oil	201224	100	<10	na
	201225	100	<10	na
	205376	100	<10	na
Complete feed	207847	34.6	24	<30
	210554	28.8	18	<30
	210555	17.0	36	<30
	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





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.conffidence.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, Deveopment and validation of a HPLC-ICPMS method for determination of methylmercury in marine food and feed, *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!