DTU Food
National Food Institute

Work package 3 - Heavy metals

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WP deputy: Matti Karp (TUT)

CON*ff*IDENCE Open Day
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SEVENTH FRAMEWORK PROGRAMME

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WP3 – partners

WP3 - outline

- Current situation in EU legislation

Foodstuffs
MLs for Pb, Cd, Hg and Sn
EU directive

Animal feedingstuffs
MLs for As, Hg, Pb, Cd and Hg
EU directive 2002/32/EC

Only maximum levels for total concentration of the metals

WP3 – trace element speciation

Information needed on

- biological activity
- toxicity
- lifetime, fate and metabolism
- chemical and physical activity

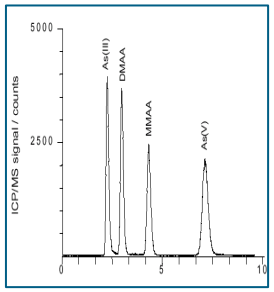
Total amount not always enough
Speciation analysis is required

The analytical speciation workhorse – HPLC-ICPMS

HPLC (GC)

Column

ICPMS



Chromatogram

WP3 – outline - analytes

- **Arsenic**
  - inorganic As (iAs) is the toxic form of As
  - feed MLs for **total As** – no regulation for food
  - lack of specific data on iAs in feed/food
  - expectation: low iAs levels

speciation

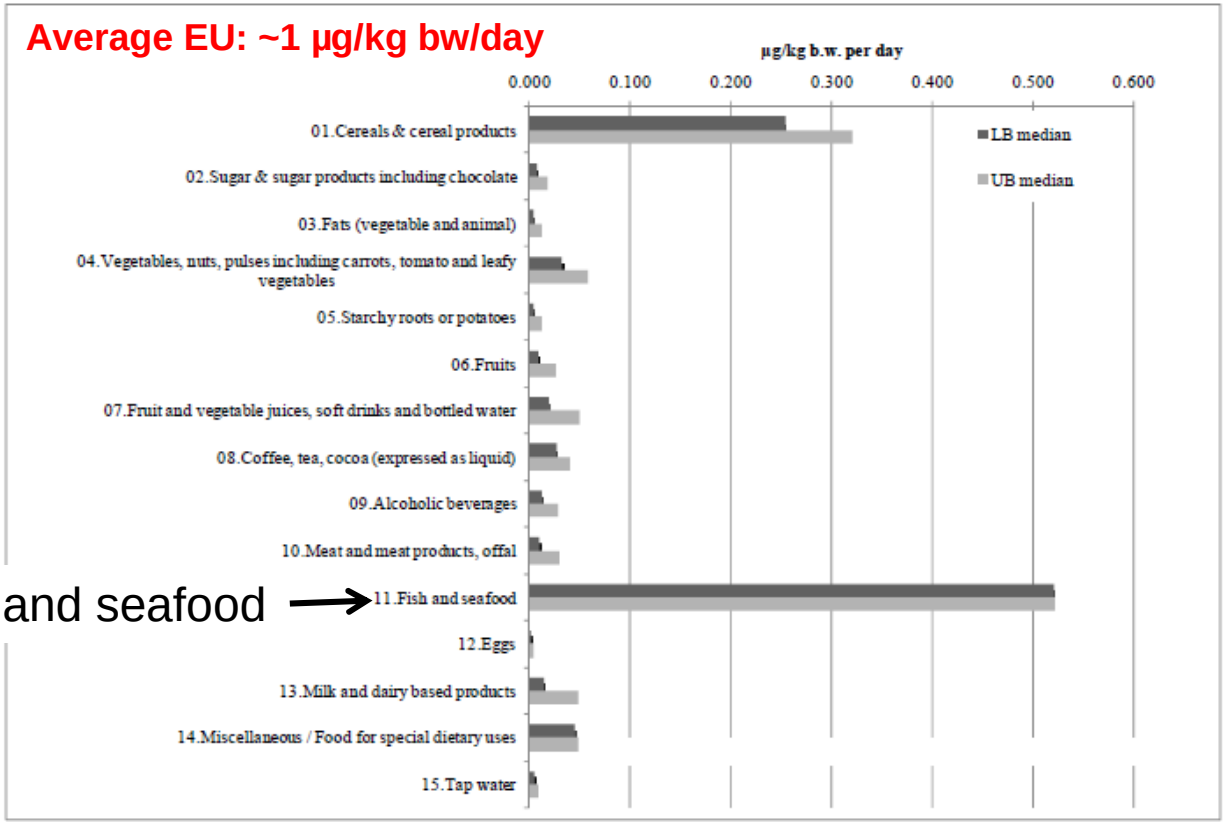
- **Mercury**
  - methylmercury (MeHg) more toxic than iHg
  - regulation on **total Hg** only
  - expectation: MeHg is the major Hg form

speciation

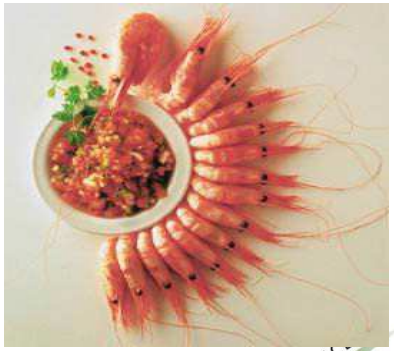
WP3 – outline – sample types

- Focus on marine feed and seafood

Seafood is the main dietary source of arsenic and mercury



EFSA, Opinion on arsenic in food, 2009.



WP3 – outline – sample types

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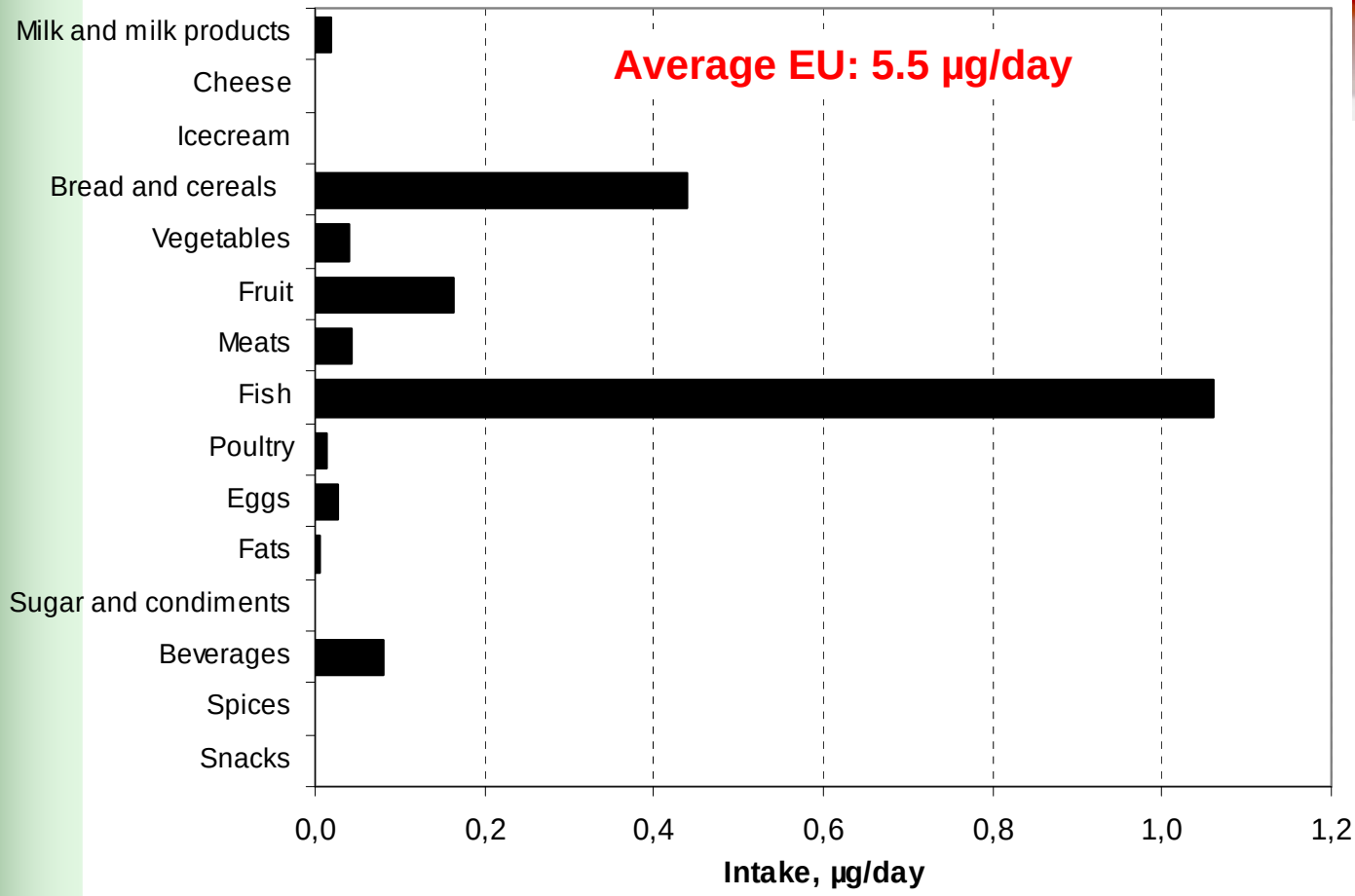
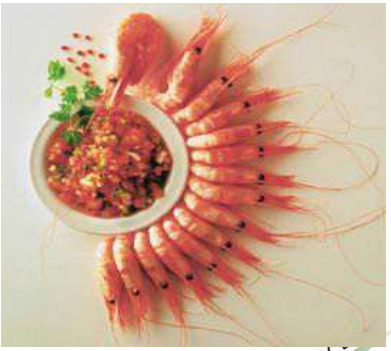




Ref: Danish Food Adm, 2004.

WP3 – objectives and tasks

- **MAE-SPE-HG-AAS**

  - simple and inexpensive speciation alternative

- **Cytosensor**

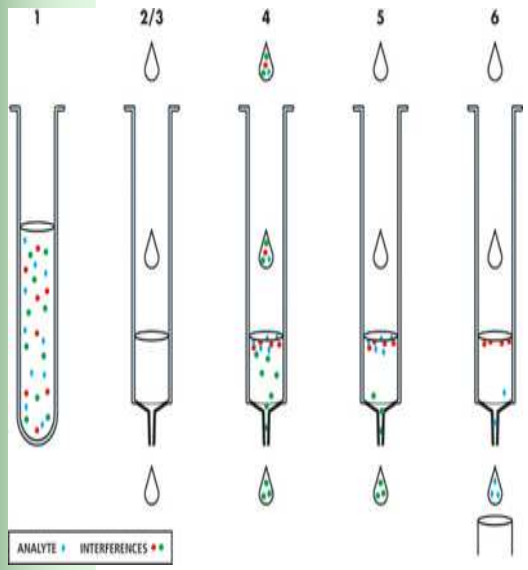
  - luminiscent recombinant bacterial cell sensor

- **Activities**

- Method development
- Validation (in-house and collaborative trial)
- Survey – data collection
- Input to risk-benefit analysis

SPE-AAS – a speciation alternative…

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



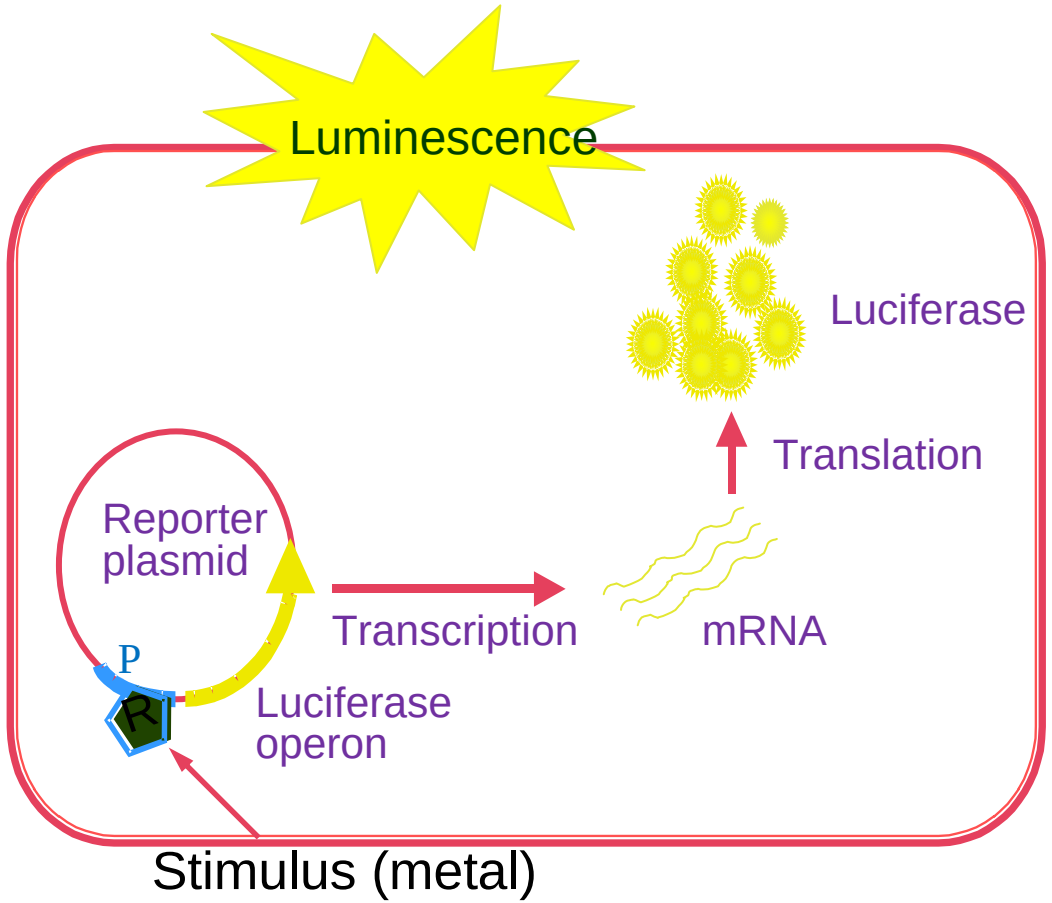
Sequential elution for selective off-line separation of inorg As from organo As species by SPE

Comparison with LC-ICPMS reference method

	SPE-HGAAS (mg/kg)	LC-ICPMS (mg/kg)
CRM TORT-2	0,94	0,95
Blue mussel	0,38	0,37
Shrimp	0,22	0,20

Principle of the luminescence-based sensor strain

specific detection of environmental factors – e.g inorganic arsenic



P =promoter

R =regulatory protein

“Components” of the metalsensor cell

- ✓ E.*coli* bacterial bioluminescent proteins are encoded by the ***lux*CDABE genes**
  - The activity of the genes is controlled by a promoter element
- ✓ The **promoter** is specific to a certain **analyte (inorganic arsenic)**
  - → The presence of inorganic arsenic triggers the activation of the bioluminescent genes
  - → Production of **bioluminescent proteins**
- ✓ Emission of **visible light** at 490 nm

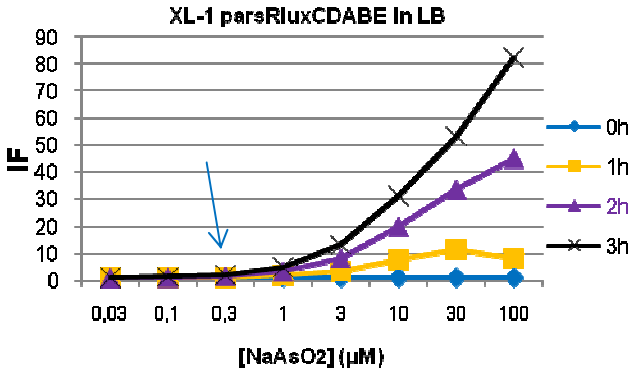
Recent results with biosensors

- Sample extraction methods for fish samples:
  - **HNO3 -extraction**
    - O/N incubation of fish sample+ nitric acid (+55 ℃, 16 h)
    - Centrifugation (13000rpm, 5 min)
    - Sample pH adjustment at 7 with phosphate buffer
    - Bioreporter assay for iAs detection
  - **Ultrasonic water -extraction**
    - Different amounts of fish sample extracted with MQ-water (10 ml)
    - Extraction performed in an ultrasonic bath (+50 ℃, 2 h)
    - Centrifugation (3000rpm, 15 min)
    - Bioreporter assay for iAs detection

→ Promising results obtained with water extraction method.

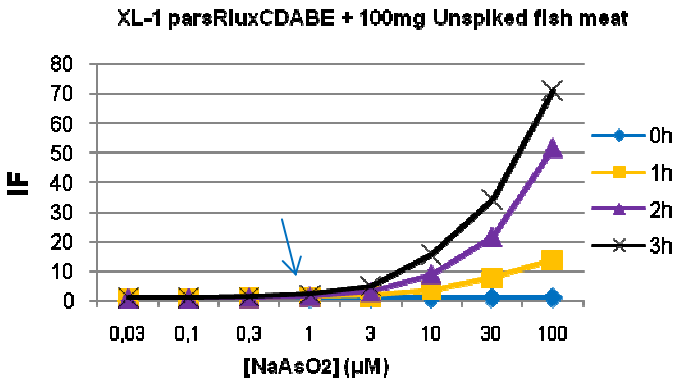
Water extraction with ultrasonic bath

- Inorganic As standard curve in LB (control)
- The detection limit is shown with the arrow



30 nM	100nM	300nM	1µM	3µM	10µM	30µM	100µM
0,85	0,84	0,90	0,91	0,90	0,89	0,89	0,89
0,95	0,96	1,16	1,79	3,40	7,42	11,25	7,85
0,95	1,09	1,58	3,16	8,11	19,73	33,46	44,81
1,06	1,36	2,14	4,90	13,19	31,39	53,19	82,30

Standard curve with 100 mg unspiked fish meat extracted with H_20[a].

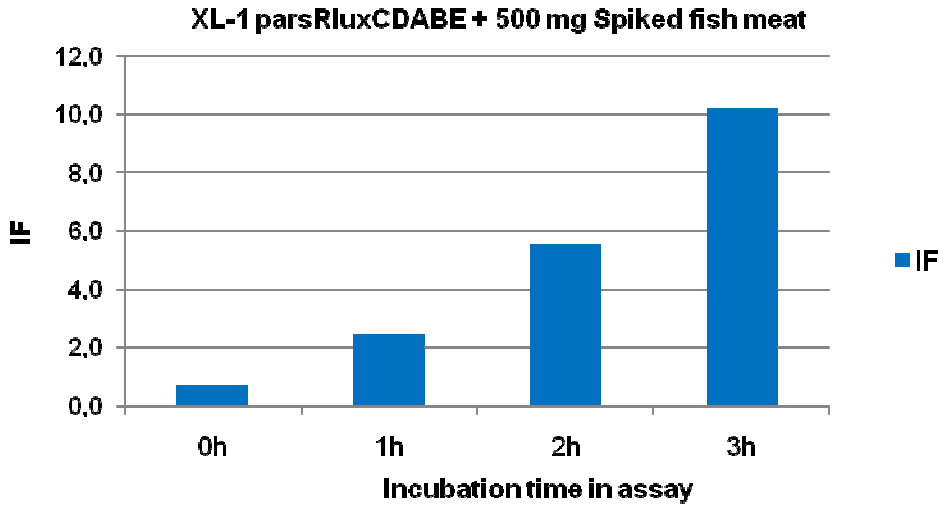


30 nM	100nM	300nM	1µM	3µM	10µM	30µM	100µM
0,93	0,92	0,93	0,96	0,95	0,97	0,99	1,06
1,02	1,01	1,06	1,24	1,66	3,47	7,76	13,88
1,09	1,09	1,20	1,72	3,05	9,04	21,76	51,62
1,10	1,11	1,36	2,29	4,97	15,87	34,43	71,27

[a] fish was cultivated without spiking, for the measurement freeze-dried fish was spiked with same concentrations of arsenic as shown in LB experiment.

Water extraction with ultrasonic bath

- **Spiked fish meat extracted with water in an ultrasonic bath (+50℃, 2 h)**



EFSA opinion on arsenic in food (issued 23/10-09)

- DATA: >100.000 occurrence data; 98% on total arsenic
- Conclusion: Old PTWI value (WHO, 1988) not appropriate any more
- NEW!: $BMDL_{01}$ = **0.3 – 8 µg/kg bw per day** for inorganic arsenic
- => EU dietary exposures within this range
- => Risk to some consumers cannot be excluded

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

- *"There is a need for robust validated analytical methods for inorganic arsenic in a range of food items"*

efsa
European Food Safety Authority

Thanks for your attention!

Further information:

Speciation – chemical analysis: **jjsl@food.dtu.dk (Jens J. Sloth)**
Speciation – biosensor approach: matti.karp@tut.fi (Matti Karp)

Various **info on speciation**: EVISA homepage; **www.speciation.net**