

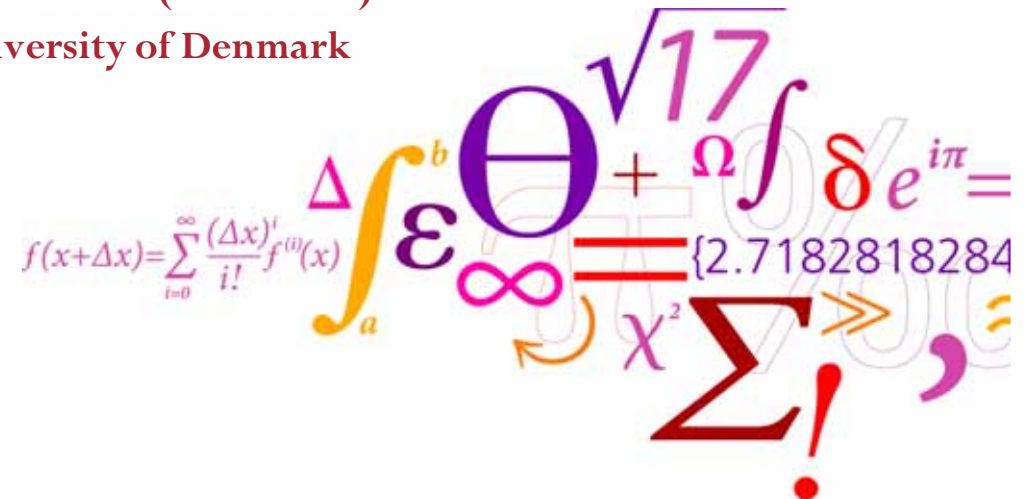


# Speciation analysis of trace elements in food and feed - status and future developments

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# AGENDA

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- **Speciation in relation to food and feed safety**
- **Selected examples**
  - 1. Arsenic speciation analysis (importance of inorganic arsenic)
  - 2. Organotin speciation analysis (food contact materials)
  - 3. Selenium speciation analysis (food fraud!)
  - .....and a little bit of nano!



# CURRENT SITUATION IN EU LEGISLATION:

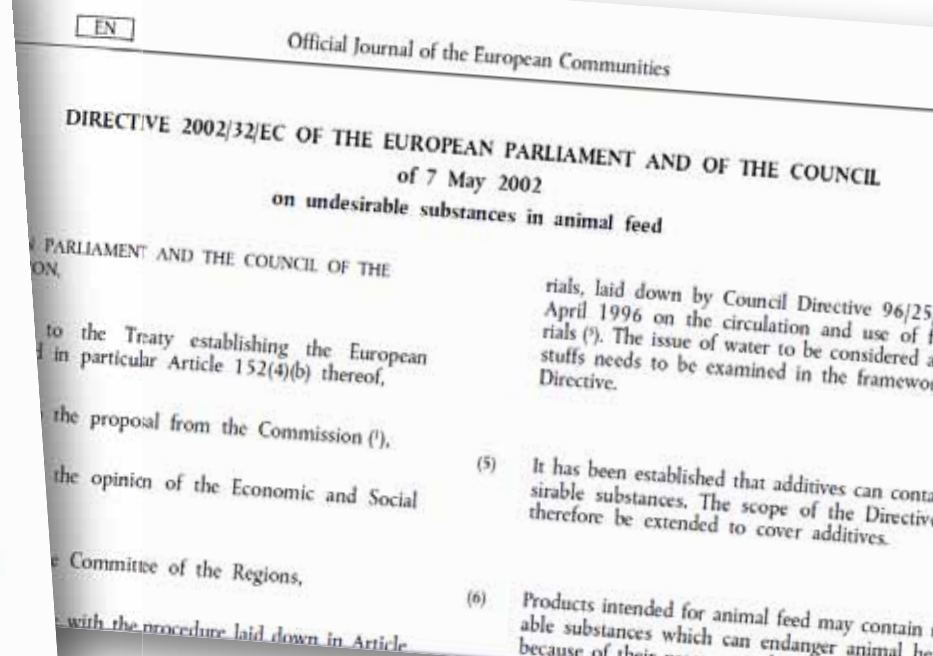
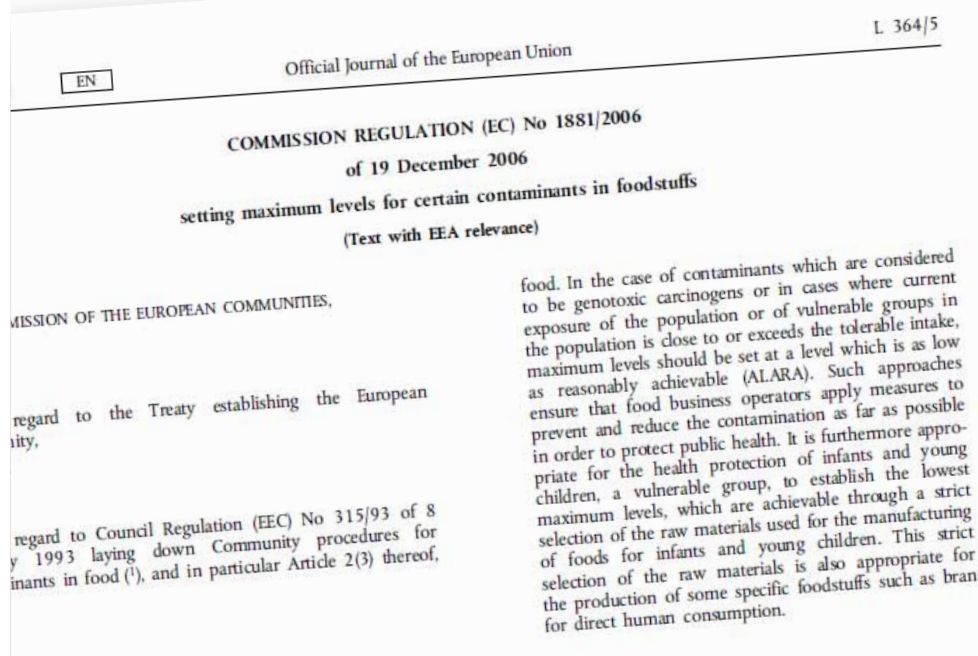
## Foodstuffs

**MLs for Pb, Cd, Hg and Sn**  
**EU directive 2006/1881/EC**

## Animal feedingstuffs

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

**Only maximum levels for**  
**TOTAL CONCENTRATION**  
**of the metals**



## Example – arsenic speciation Important for correct risk assessment



**~ 40 – 250  $\mu\text{g}$  inorg As**

**1 kg rice => 50-300  $\mu\text{g}$  As**



**<10  $\mu\text{g}$  inorg As**

**1 kg fish => 3000-10000  $\mu\text{g}$  As**




**There is most focus on rice from a food safety point of view – why???**



**The chemical form of arsenic is important  
=> Arsenic speciation**

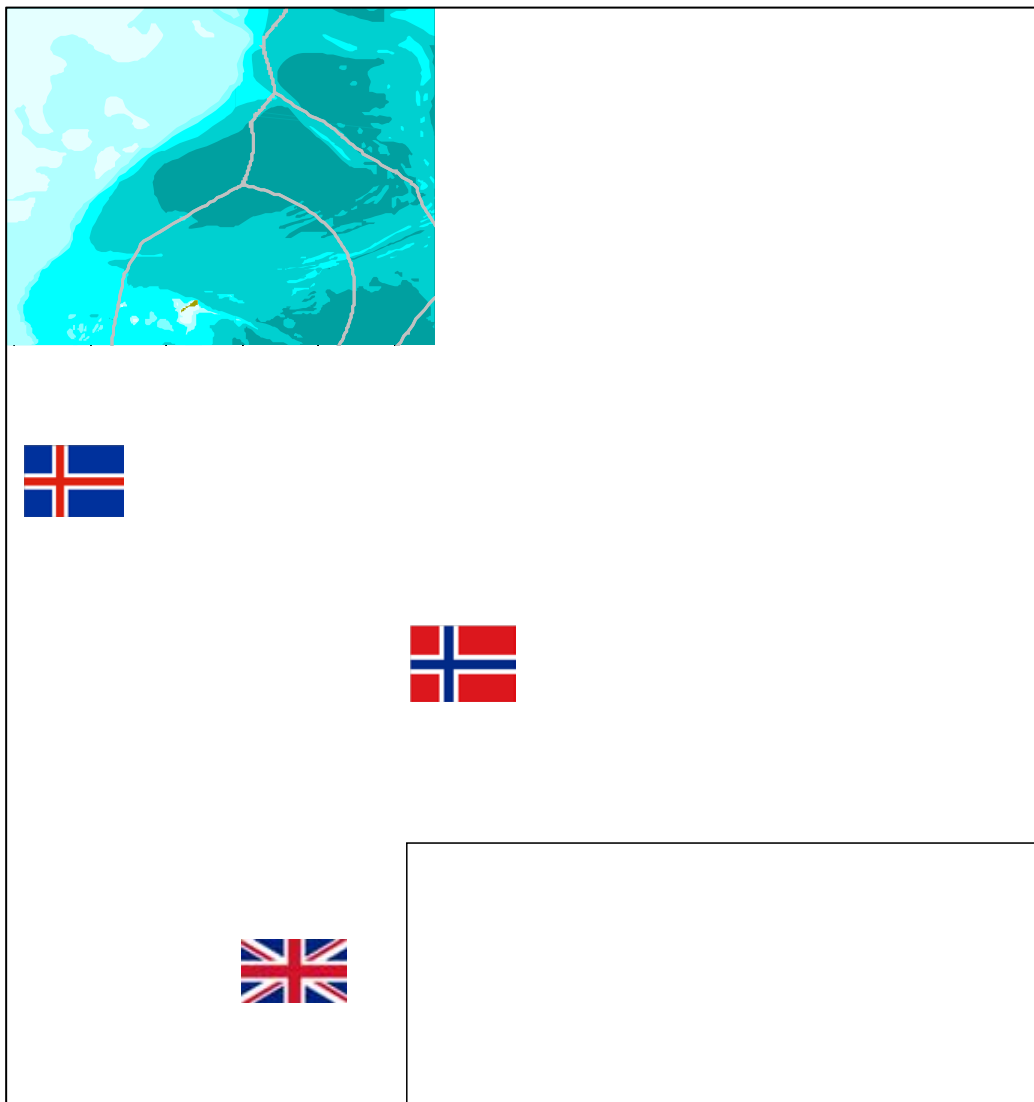
## EFSA (2009) and JECFA (2010) opinions on arsenic in food

- Old PTWI value (WHO, 1988) was withdrawn ( $15 \mu\text{g}/\text{kg bw}/\text{week}$ )

 <p>European Food Safety Authority</p>	<ul style="list-style-type: none"> <li>• <b>NEW!</b> <math>\text{BMDL}_{1.0} = 0.3 - 8 \mu\text{g}/\text{kg bw}</math> per day for inorganic arsenic</li> <li>• =&gt; EU dietary exposures within this range for average and high level consumers</li> <li>• =&gt; Risk to some consumers cannot be excluded</li> </ul>
 	<ul style="list-style-type: none"> <li>• <b>NEW!</b> <math>\text{BMDL}_{0.5} = 3 \mu\text{g}/\text{kg bw}</math> per day for inorganic arsenic =&gt; 0.5% increased incidence of lung cancer for 12 y exposure</li> </ul>

- “...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”

# Inorganic arsenic in wild caught fish => no concern



## Norwegian survey

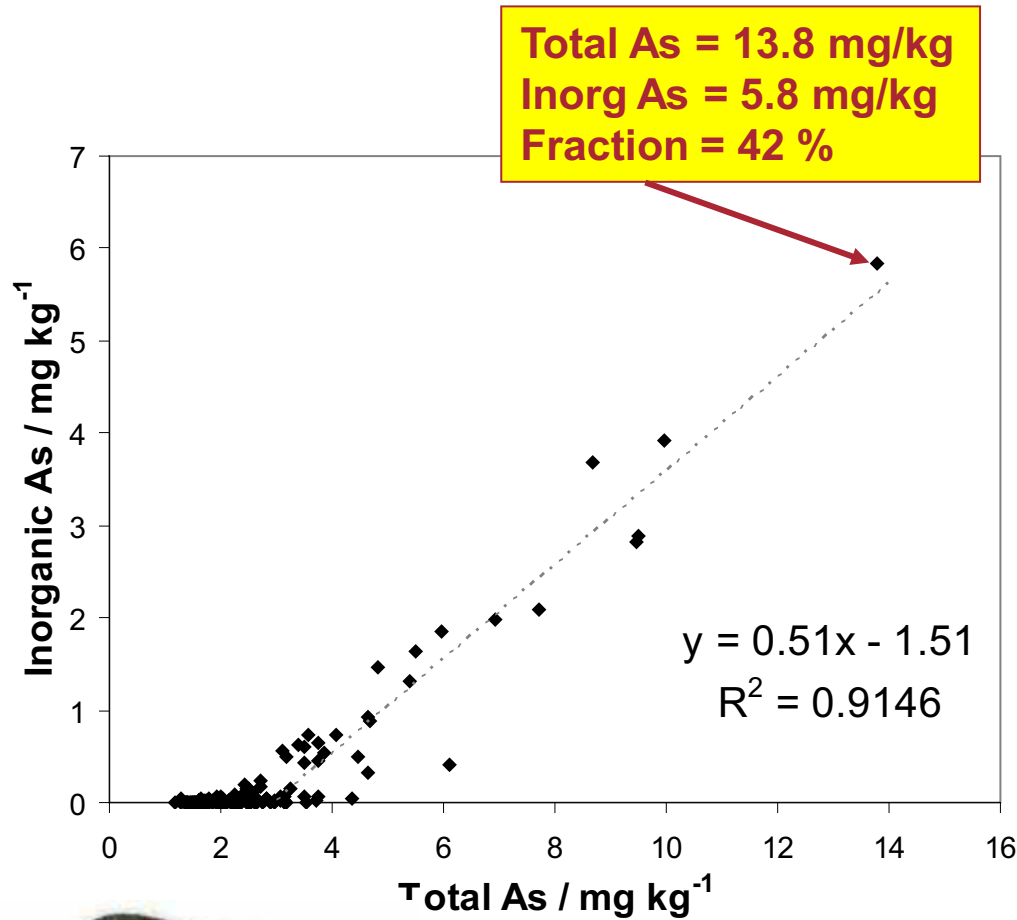
900 individual fish samples

- 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)*

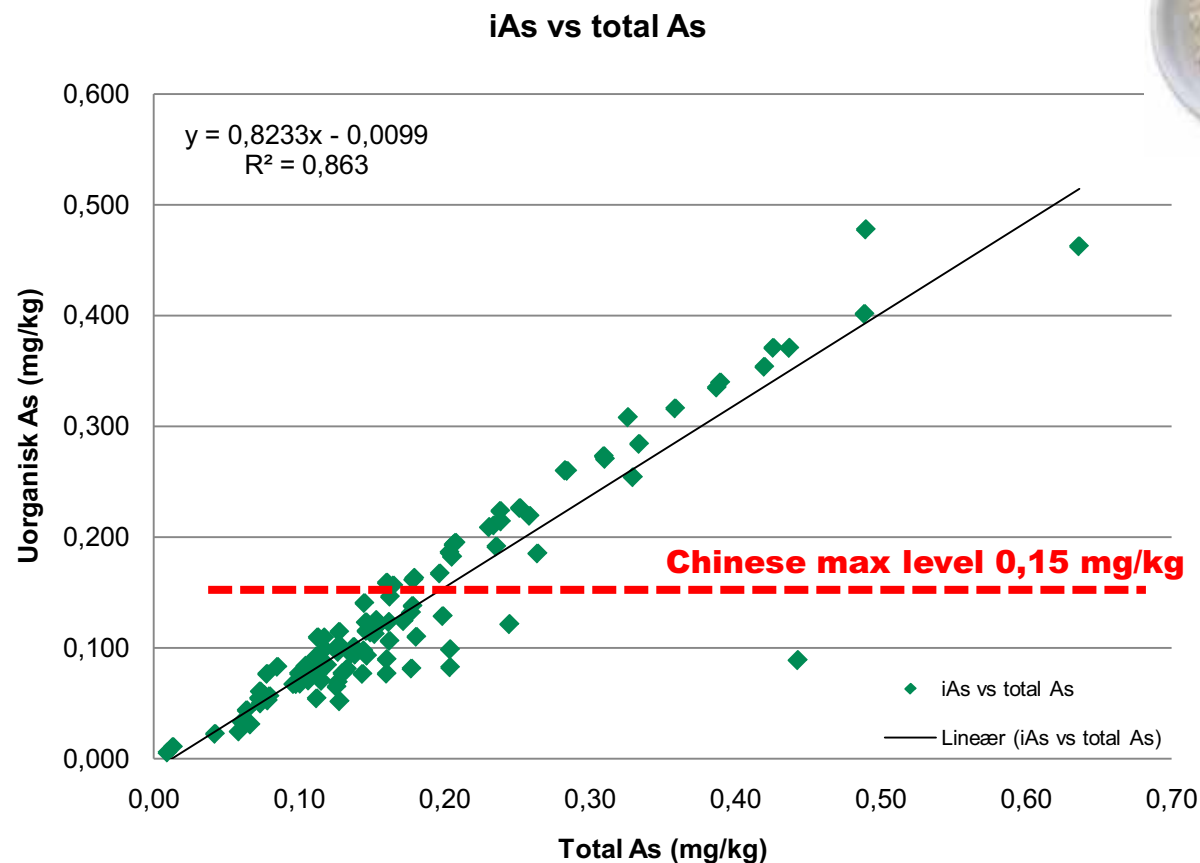
# ...but in bivalves high contents in some samples...



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



# Arsenic in rice products DK - survey



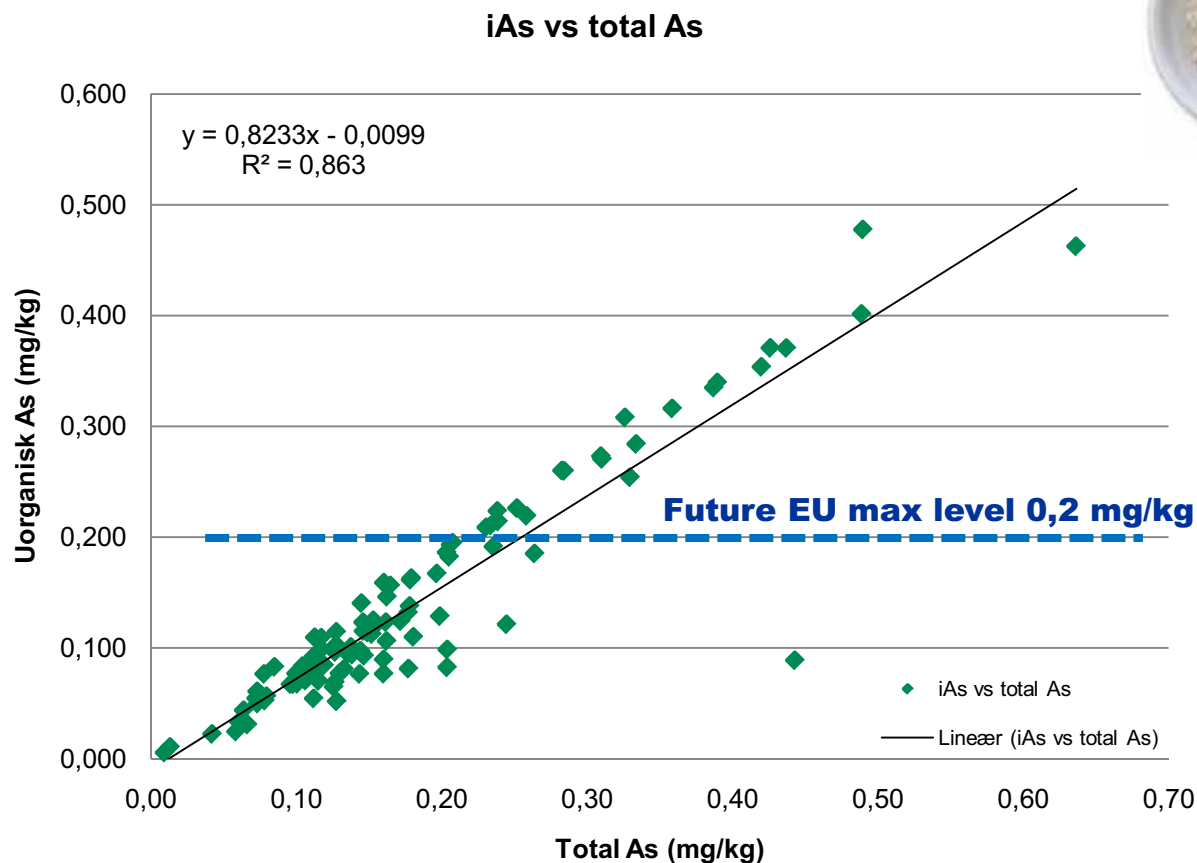
## **105 samples**

- white rice**
- brown rice**
- red rice**
- black rice**
- rice crackers**

## **33 samples > 0,15 mg/kg**

- 2 parboiled (20%)**
- 4 brown (50%)**
- 4 red (50%)**
- 5 black (71%)**
- 1 Basmati (10%)**
- 1 Pudding rice (9%)**
- 1 wild rice (20%)**
- 15 rice crackers (100%)**

# Arsenic in rice products DK - survey



## 105 samples

- white rice
- brown rice
- red rice
- black rice
- rice crackers

## 22 samples > 0,2 mg/kg

- 1 parboiled (10%)
- 1 brown (12%)
- 3 red (37%)
- 2 black (28%)
- 0 Basmati (0%)
- 0 Pudding rice (0%)
- 0 wild rice (0%)
- 15 rice crackers

Rice cracker mean: 0.31 mg/kg – intake 50 g/dag => 15 µg iAs (~1 µg/kg bw/dag)

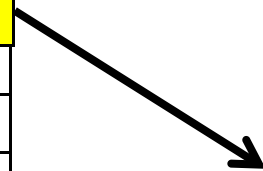
> EFSA BMDL<sub>01</sub> 0.3-8 µg/kg bw/dag



# Inorganic arsenic in chinese food supplements



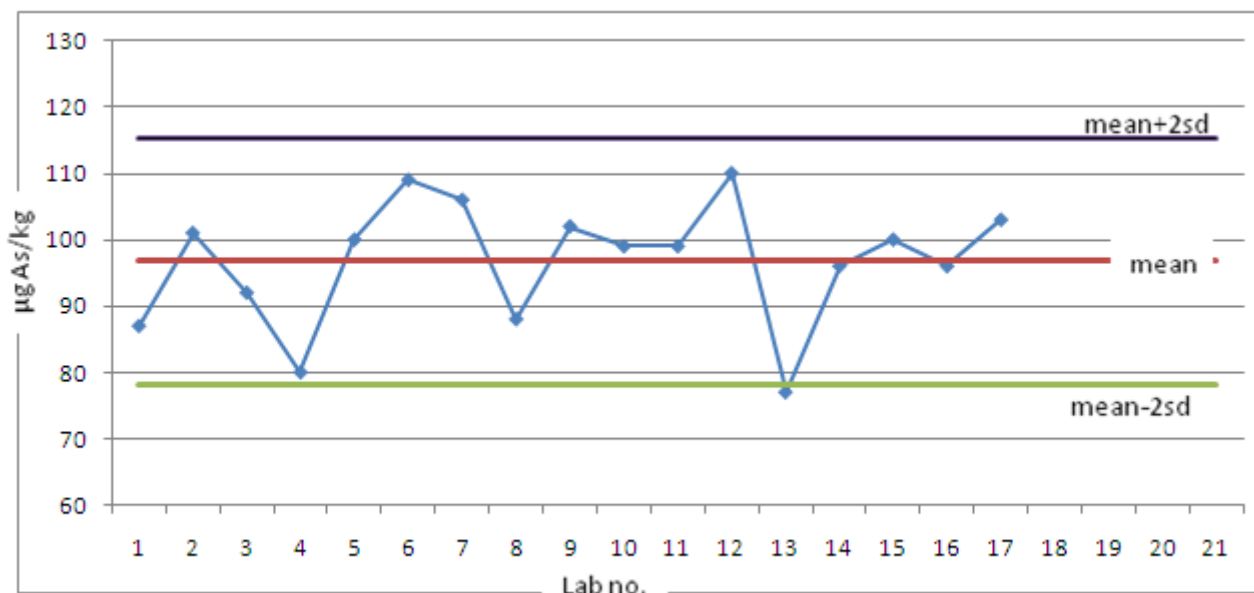
Name of Food supplement	Total Arsenic ( $\mu\text{g/g}$ )	Inorganic arsenic ( $\mu\text{g/g}$ )
Xiao Yao Wan	0.82	0.85
Bu Zhong Yi Qi Wan	0.62	0.50
Da Bu Yin Wan	0.59	0.55
Six Flavor teapills	0.72	N.D.
Golden Book Teapills	0.58	0.57
Xiang Sha Liu Jun Zi Wan	0.94	0.80
<b>Gan Mao Ling</b>	<b>1.24</b>	<b>1.01</b>
Chuan Xin Lian	5.00	3.17
Bi Yan Pian	0.70	0.58
Arouse power	1.12	1.02
Bio Chlorella	0.62	0.21
Unik Spirulina Kapsler	2.59	0.13
Chlorella	0.58	0.03
Ez-Biloba	0.63	0.67
Qvinde Dong Quai	0.68	0.48



**Gan Mao Ling**  
**(against flu and common cold)**

**Rec dose: 18 pills per day**  
 $\Rightarrow$  **iAs ~ 13  $\mu\text{g/day}$**   
 $\Rightarrow$  **0.22  $\mu\text{g/kg bw/day}$  (@60 kg)**  
**Close to EFSA BMDL<sub>01</sub> !!**

# Inorganic As in SRM NIST1586a – literature data



**Mean =  
0.097 mg/kg**

Extraction solution	Detection	iAs (µg/kg)	Reference
1 2M TFA	LC-ICPMS	87 +/- 9	Ackermann (2005)
2 Enzymatic, pepsin and pancreatin	LC-ICPMS	101 +/- 7	Ackermann (2005)
3 2M TFA	LC-ICPMS	92 +/- 2	Heitkemper (2009)
4 2M TFA	LC-ICPMS	80 +/- 16	Williamson (2008)
5 2M TFA	LC-ICPMS	100 +/- 10	Williamson (2008)
6 MeOH:H2O	LC-ICPMS	109 +/- 3	D'Amico (2008)
7 Enzymatic, alfa-amylase	LC-ICPMS	106 +/- 7	Kohlmeier (2008)
8 Enzymatic, protease and alfa-amylase	LC-ICPMS	88 +/- 6	Sanz (2009)
9 1M H3PO4	HG-AFS	102 +/- 2	Matosreyes (2008)
10 1% HNO3	LC-ICPMS	99 +/- 4	Raab (2009)
11 1% HNO3	LC-ICPMS	99 +/- -	Sun (2008)
12 1% HNO3	LC-ICPMS	110 +/- 10	Sun (2009)
13 0,5 M TFA	LC-ICPMS	77 +/- -	Heitkemper (2009)
14 enzymatic, proteas and alfa-amylase	LC-ICPMS	96 +/- 9	Mar (2009)
15 2M TFA	LC-ICPMS	100 +/- 12	Meharg (2008)
16 water	LC-ICPMS	96 +/- 3	Narukawa (2008)
17 0,07M HCL and 10%H2O2	LC-ICPMS	103 +/- 15	DTU Food (2009)

**Good agreement  
between  
labs and methods**

# Animal feedingstuffs

## Commission directive 2009/114/EC (amendment)

Undesirable substances	Products intended for animal feed	Maximum content in mg/kg (ppm) relative to a feedingstuff with a moisture content of 12 %
(1)	(2)	(3)
1. Arsenic (*) (**)	Feed materials with the exception of:	2
	— meal made from grass, from dried lucerne and from dried clover, and dried sugar beet pulp and dried molasses sugar beet pulp,	4
	— palm kernel expeller,	4 (***)
	— phosphates and calcareous marine algae,	10
	— calcium carbonate,	15
	— magnesium oxide,	20
	— feedingstuffs obtained from the processing of fish or other marine animals, including fish,	25 (***)
	— seaweed meal and feed materials derived from seaweed,	40 (***)
	Iron particles used as tracer,	50
	Additives belonging to the functional group of compounds of trace elements except:	30
Complete feedingstuffs with the exception of:	Complete feedingstuffs with the exception of:	2
	— complete feedingstuffs for fish and complete feedingstuffs for fur animals,	10 (***)
	Complementary feedingstuffs with the exception of:	4
— mineral feedingstuffs,	12	



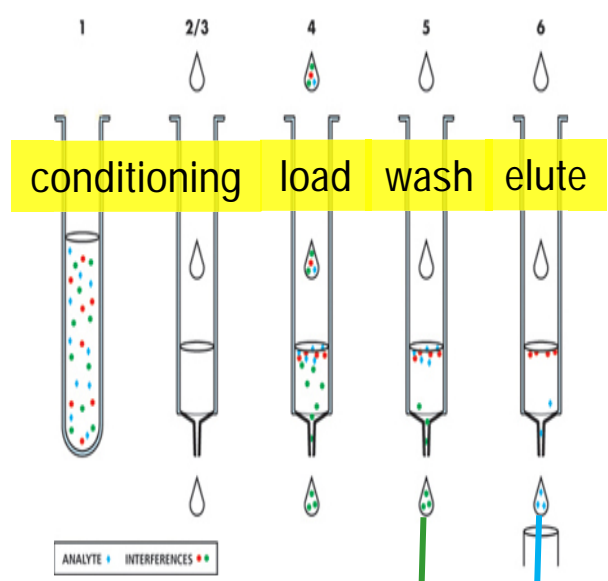
**Only max levels for total arsenic**

### FOOTNOTE

(\*\*\*) Upon request of the competent authorities, the responsible operator must perform an analysis to demonstrate that the content of inorganic arsenic is lower than 2 ppm in feedingstuffs of particular importance for the seaweed species *Hizikia fusiforme*.

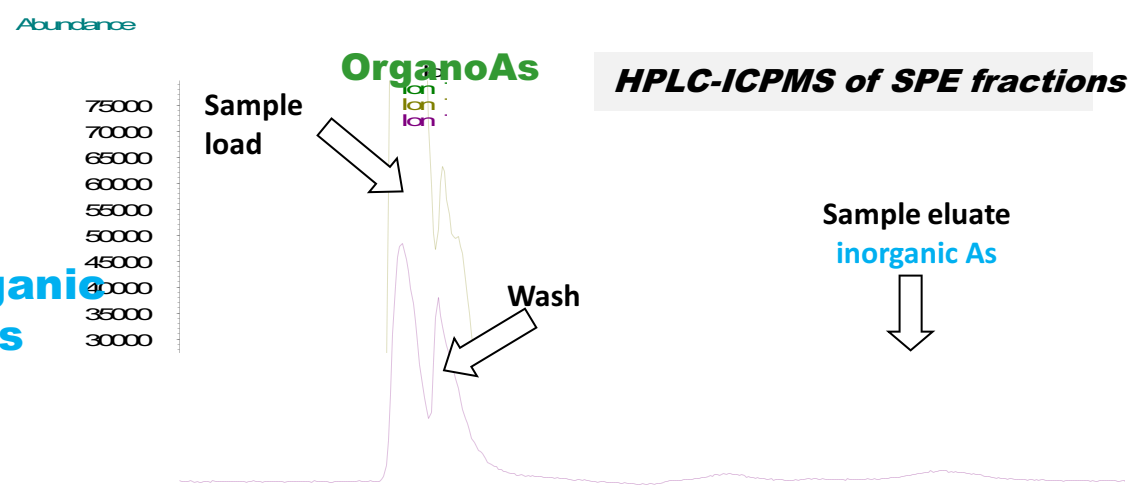
Speciation analysis is required !!

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



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

**OrganoAs compounds**  
**Inorganic As**



# SPE-HG-AAS

μ-wave extraction

Separation by  
SPE

Detection by HG-  
AAS



## In-house validation data



Parameter	Result
Analysis time	2 x 7 h for 24 samples
LoD (mg/kg)	0.08
LoQ (mg/kg)	0.16
Repeatability (%RSD)	3 - 7
Accuracy (%)	90 - 104

## Collaborative trial in marine feed material

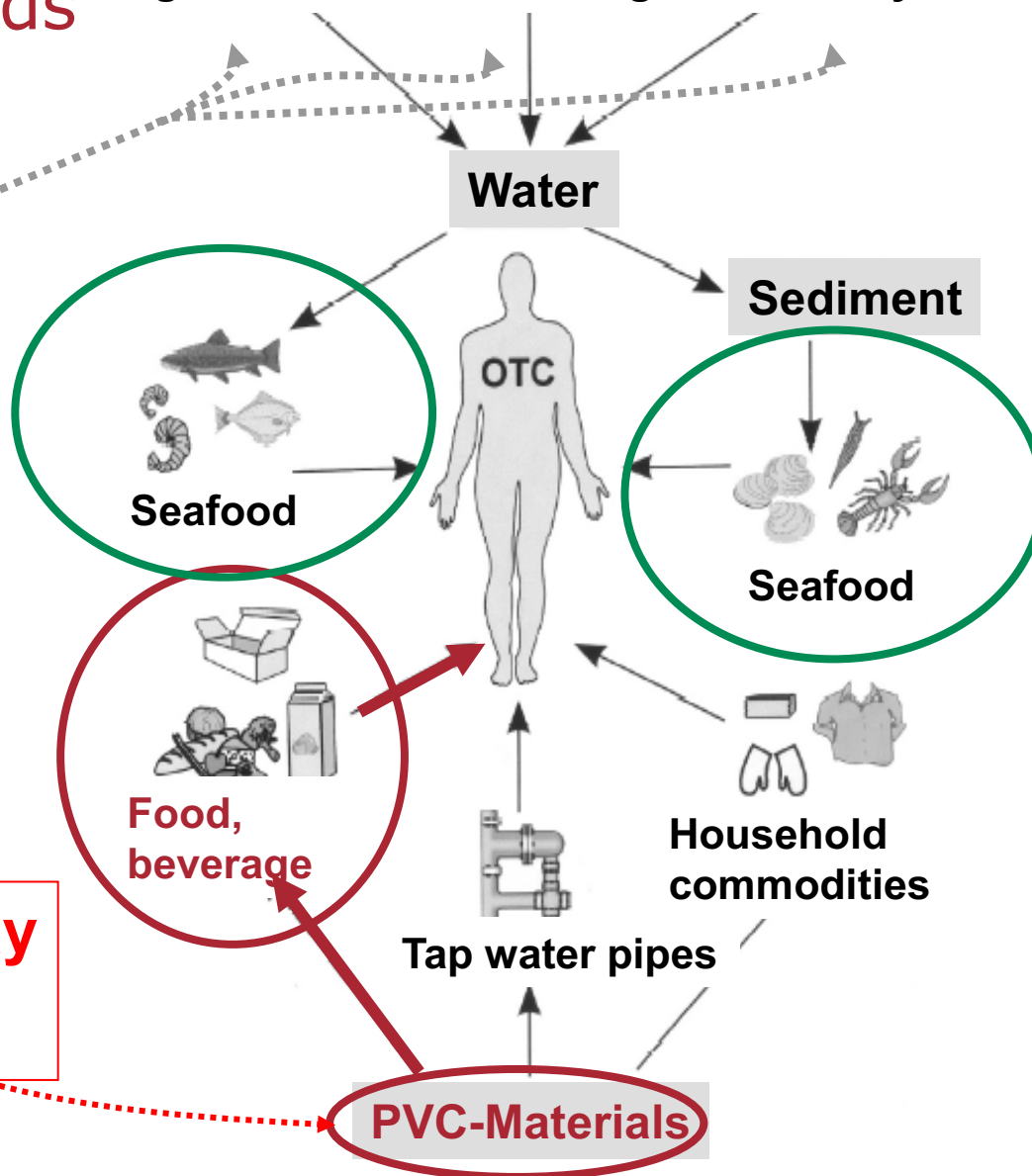


- **10 labs provided results**
- **Method working range tested: 0.1 – 2.6 mg Kg<sup>-1</sup>**
- **HorRat values <2 in the working range tested**
- **The method is fit for purpose**
- **Final publication as EN expected July 2012**

# Routes of exposure to organotin compounds

Used in

- Agriculture
- Antifoulings
- Industry



- PVC-Materials

**TDI: 0.25  $\mu\text{g}/\text{kg bw}/\text{day}$**   
 $\sum$  TBT, DBT, TPhT and DOT

# Legislation on OTCs in Food Contact Materials



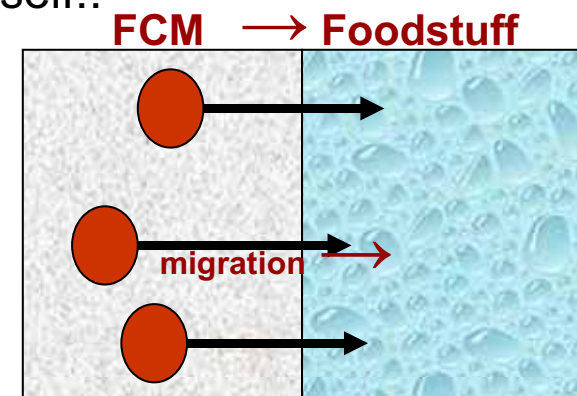
Compounds	Maximum level ( $\mu\text{g Sn/kg}$ foodstuff)
$\sum$ DBT, TBT, TPhT and DOT	40 (6)
$\sum$ MMT, DMT	180
MOT	1200
MDDT	12000 (50)
DDDT	24000 (50)

## Assumptions:

- 1 kg food per 6 dm<sup>2</sup>
- 100 mL in contact with 0.6 dm<sup>2</sup>

Ref: EFSA (2005); proposed EFSA values in parenthesis

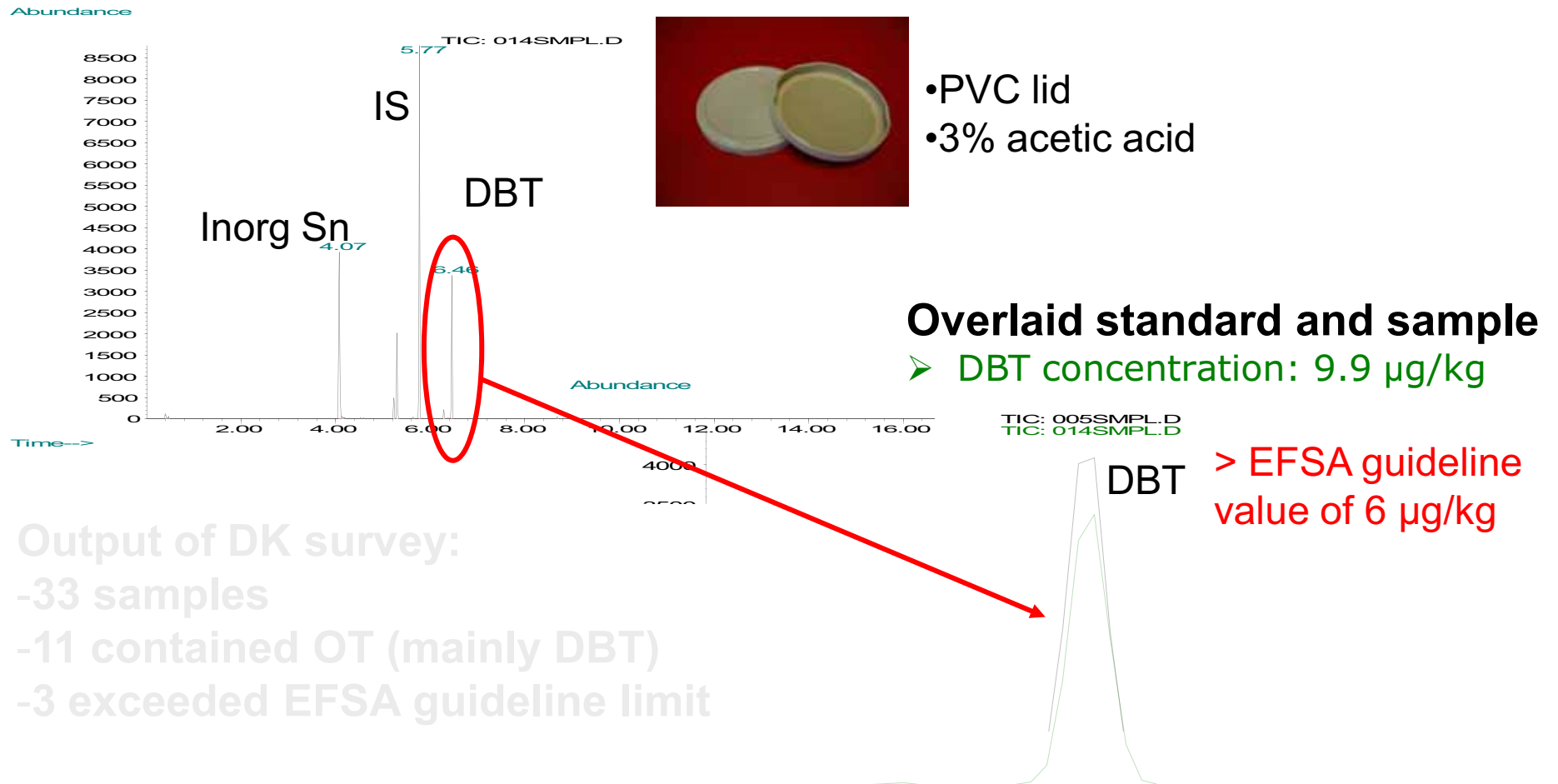
- Max levels on organotin migrating from the packaging material
- Testing by the use of food simulators (water, acid, oil, alcohol etc)
- BUT no maximum levels on organotins in the foodstuff itself!!



# Organotin migration from Food Contact Materials II

## Small scale survey on 33 FCMs

Baking paper, PVC cling films, silicone baking forms, lids with PVC gaskets  
PUR-agglomerated cork wine stoppers



Output of DK survey:  
-33 samples  
-11 contained OT (mainly DBT)  
-3 exceeded EFSA guideline limit

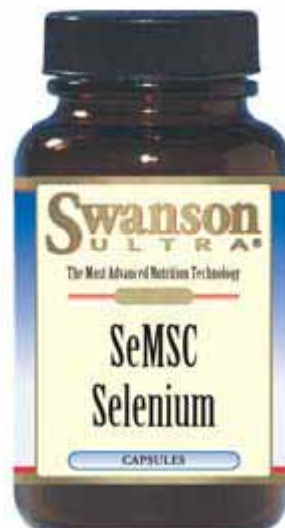
# Selenium in commercial food supplements

*Organic bound Se?*



*Selenite?*

*Selenate?*



*Se yeast?*

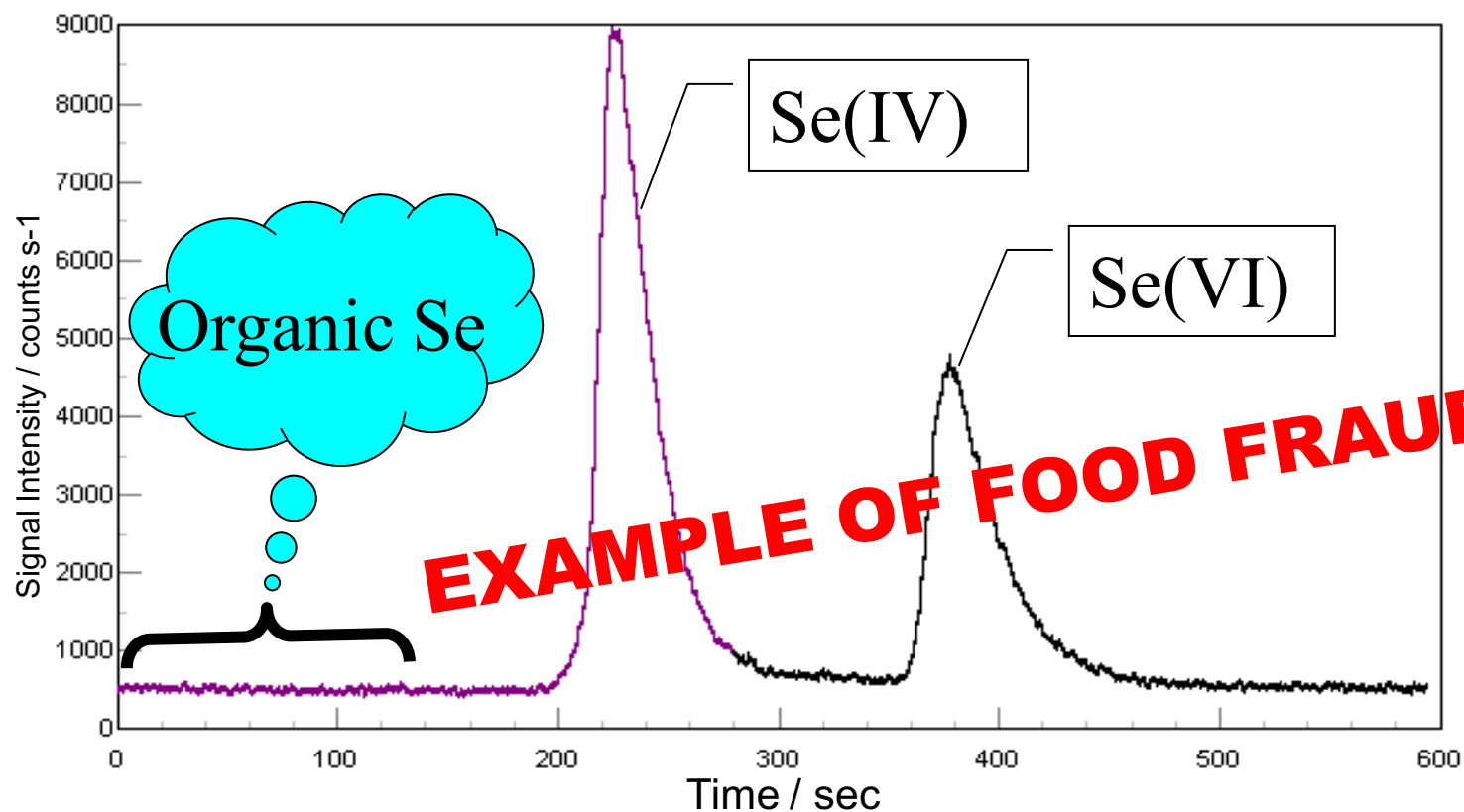
*Selenomethionine?*

*Amino chelated Se?*

**Is the selenium source declared correctly??**

# Se speciation by HPLC-ICPMS

Supplement declared as organic bound Selenium (125  $\mu\text{g}$ /tablet)



## Speciation summary

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- ✓ speciation methods are more and more commonly used
- ✓ instrumentation is widely available
- ✓ legislation on species has started
- ✓ ...and more is expected in the future!



- ✓ standardised methods are not ready!
- ✓ ...but the need is known by authorities
- ✓ legislation is still behind!
- ✓ Lack of CRMs (e.g. for iAs)

# Perspective – trace elements in nanoform

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## NanoLyse Project "Nanoparticles in Food: Analytical methods for detection and characterisation"

Validated methods for the determination of inorganic ENP in food extracts, based on size separation, size determination and specific detection

### ***silver nanoparticles in lean meat***

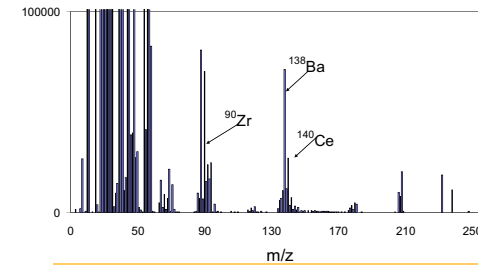
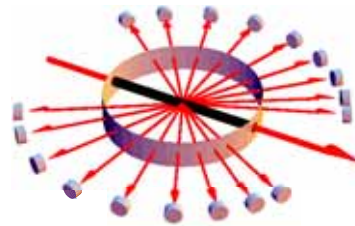
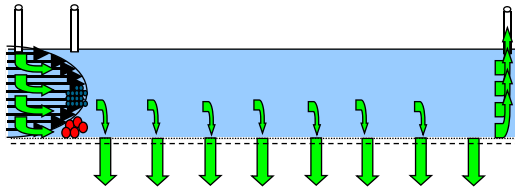


### ***silica nanoparticles in tomato soup***



**<http://www.nanolyse.eu>**

# DTU Food Analytical platform: AF4-MALS/DLS-ICP-MS



**asymmetric flow  
field flow  
fractionation  
(AF4)**

**optical detection  
multi angle (MALS)  
and dynamic light  
scattering (DLS), UV-  
vis absorption and  
fluorescence**

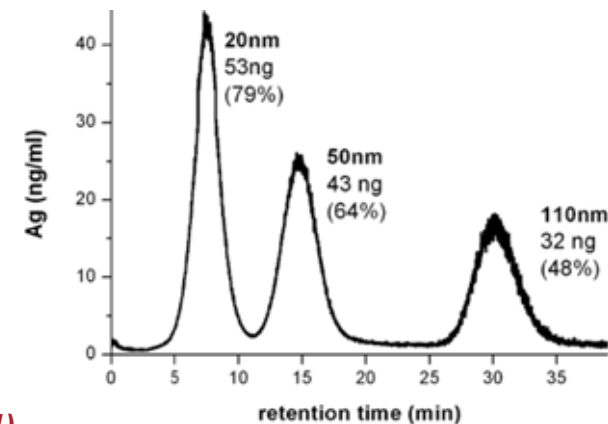
**inductively coupled  
plasma mass  
spectrometry  
(ICP-MS)**

**particle separation  
according to their  
size (1nm – few  $\mu\text{m}$ )**

**particle detection  
size determination**

**elemental detection  
chemical identity  
quantification**

Fractogram =>





## Acknowledgements and funding sources

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ANNUAL MEETING & EXPOSITION



# Thanks for your attention!

