

## Speciation of Heavy Metals - an important parameter for risk assessment of feed and food safety in aquaculture

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# SPECIATION OF HEAVY METALS

– AN IMPORTANT PARAMETER FOR RISK ASSESSMENT OF FEED AND FOOD SAFETY IN AQUACULTURE

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*National Food Institute*

*Technical University of Denmark*



[www.confidence.eu](http://www.confidence.eu)



# Current situation in EU legislation:

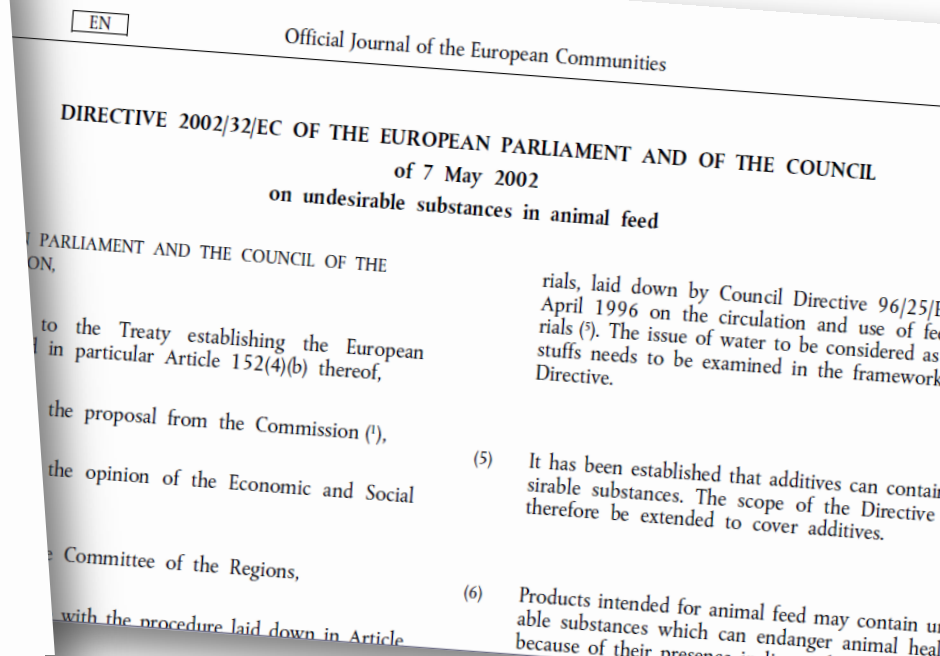
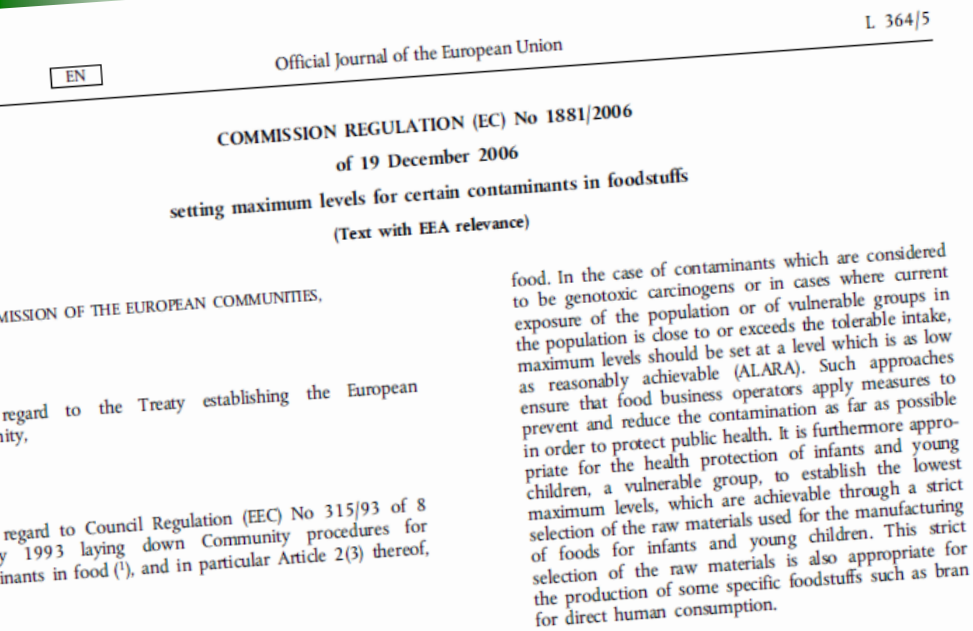
## Foodstuffs

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**

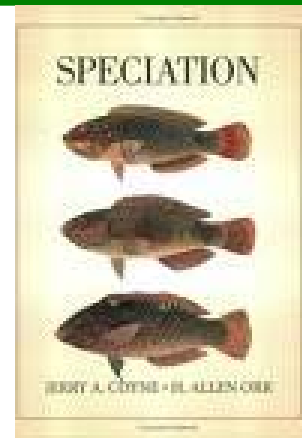


# Speciation of elements

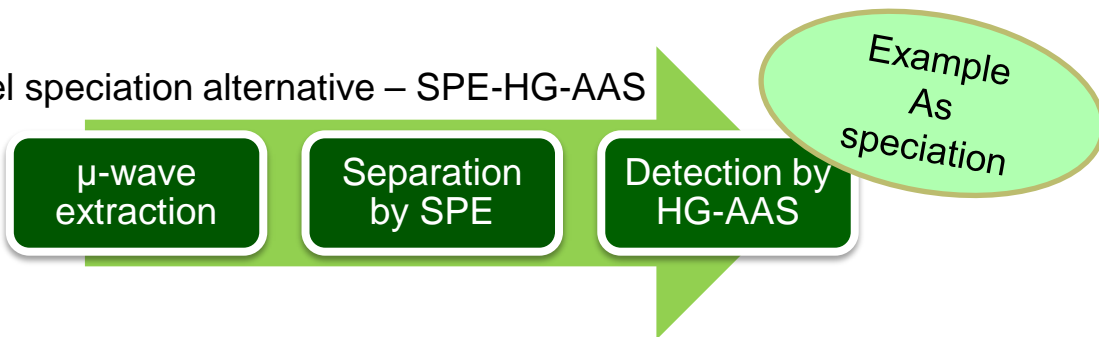
Information needed on

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

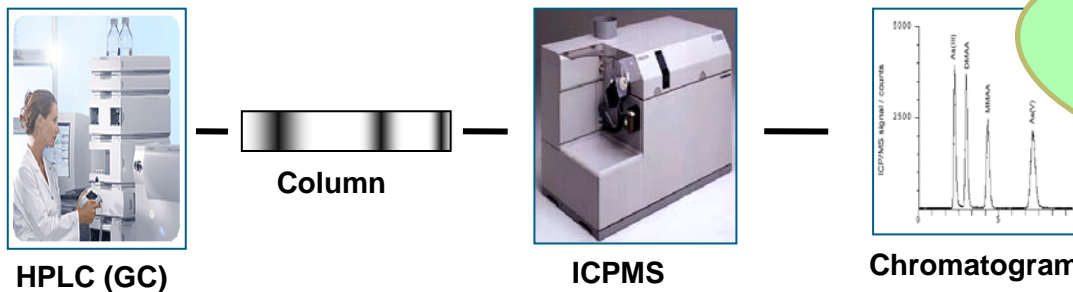
Total amount not always enough  
Speciation analysis is required



Novel speciation alternative – SPE-HG-AAS



The analytical speciation workhorse – HPLC-ICPMS



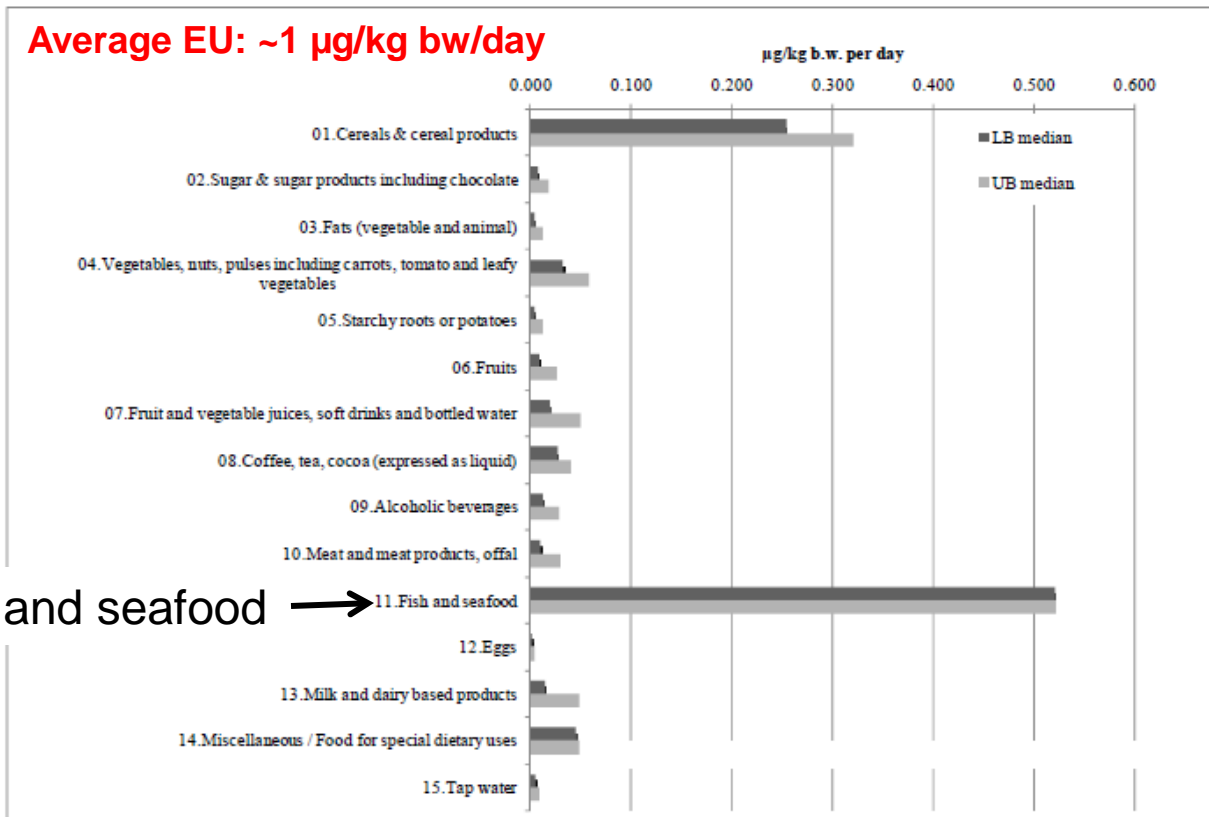
Example Hg speciation



# Selected sample types

## ➤ Focus on marine feed and seafood

Seafood is the main dietary source of **arsenic** and mercury



Fish and seafood →

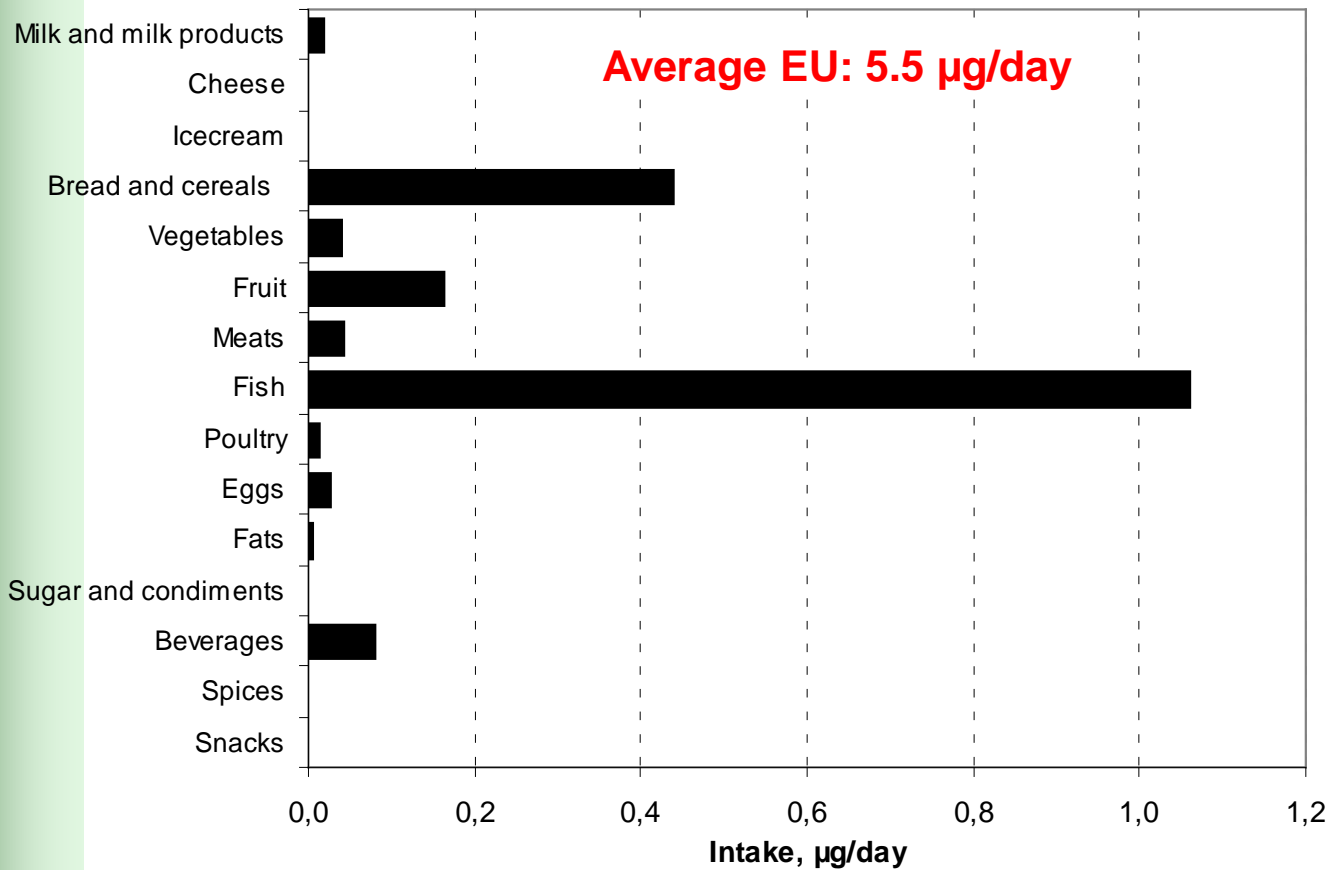
EFSA (2009), Scientific Opinion on Arsenic in Food



# Selected sample types

## ➤ Focus on marine feed and seafood

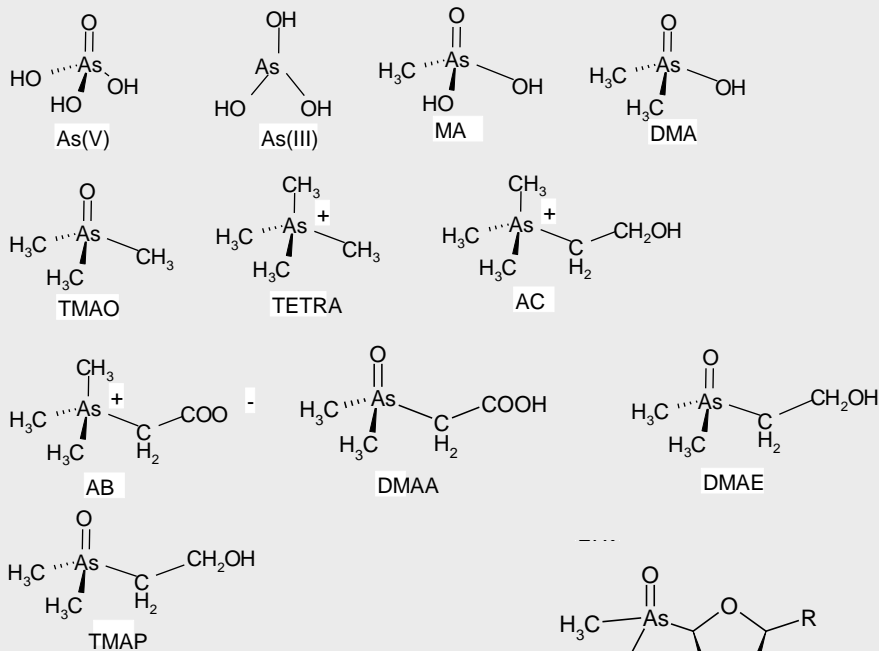
Seafood is the main dietary source of arsenic and **mercury**



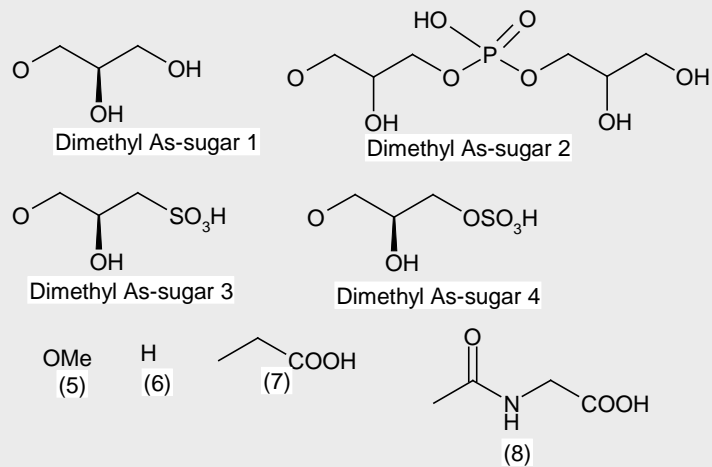
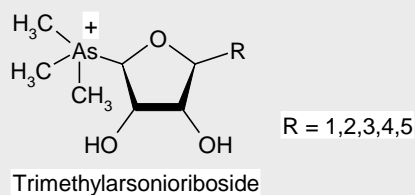
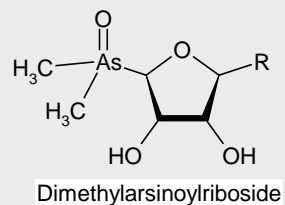
Ref: Danish Food Adm, 2004.



# Arsenic compounds in the marine environment



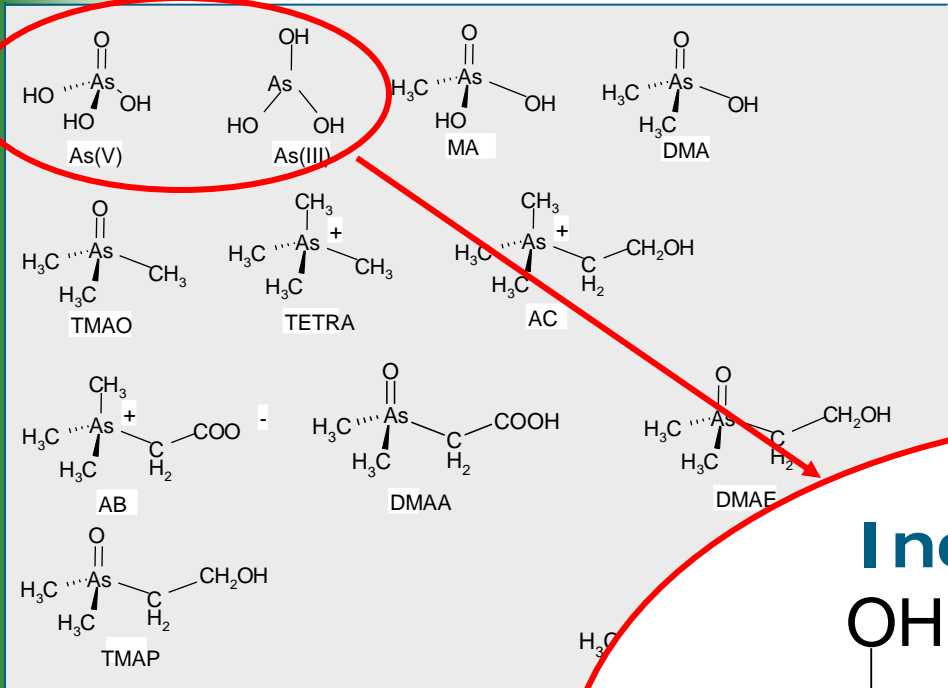
More than **50** different arsenic species have been found in the marine environment



# Arsenic compounds in the marine environment

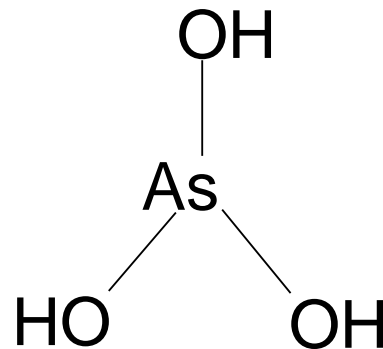


Arsenocosis

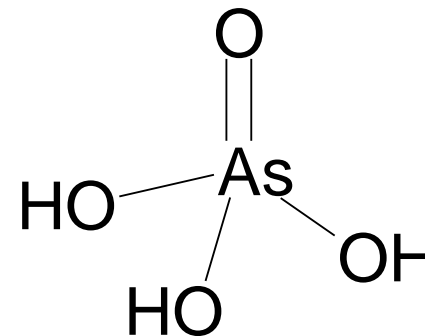


**Most toxic form of arsenic!!**

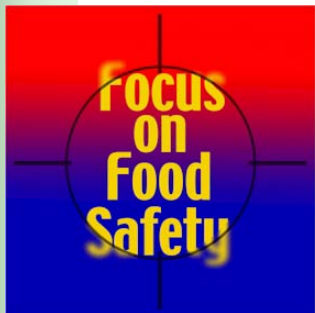
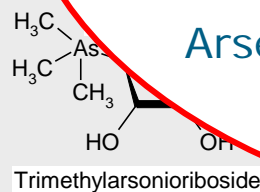
## Inorganic arsenic



Arsenous acid  
As(III)

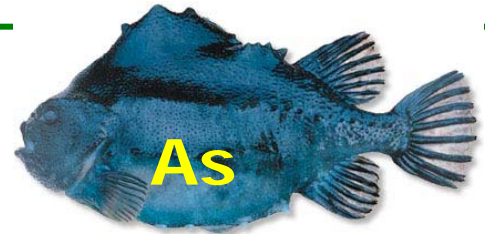


Arsenic acid  
As(V)

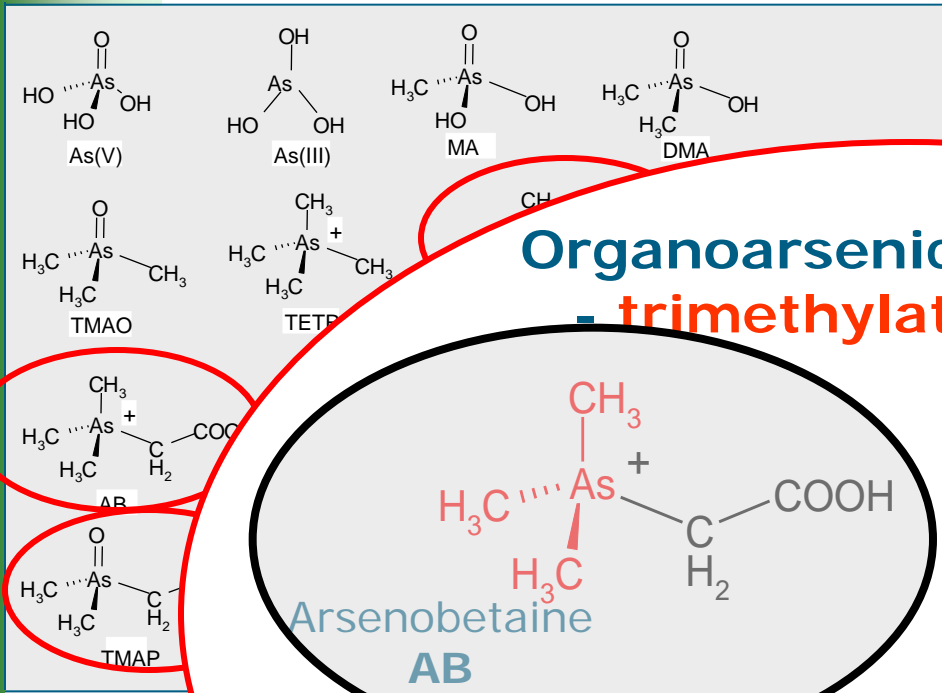




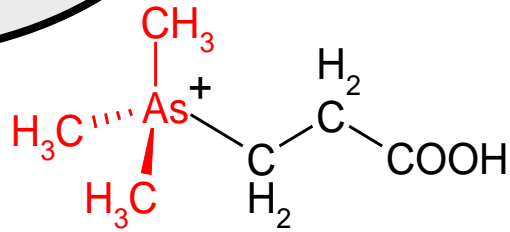
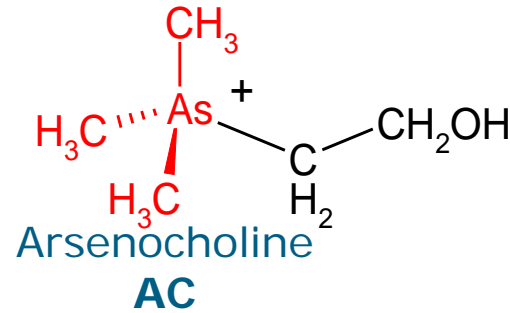
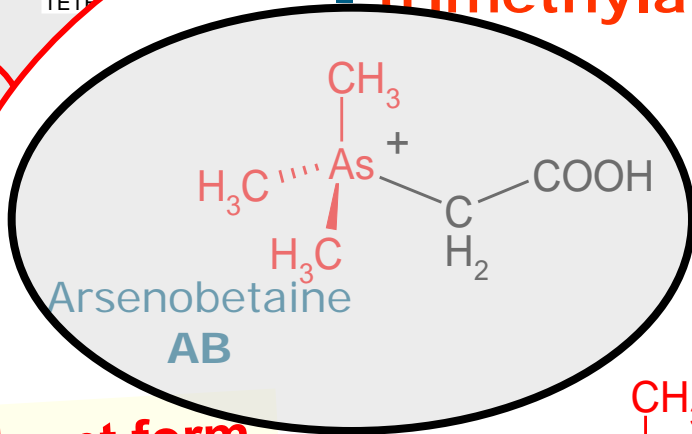
# Arsenic compounds in the marine environment



AB="fish arsenic"  
(Chapman, Analyst, 1920)



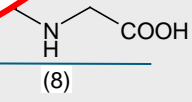
## Organoarsenic compounds - trimethylated species



Trimethylarsoniopropionic acid  
**TMAP**

**Predominant form of arsenic in most seafood!!**

Trimethylarsonioriboside



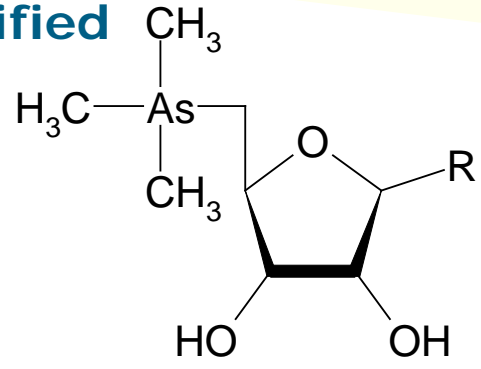
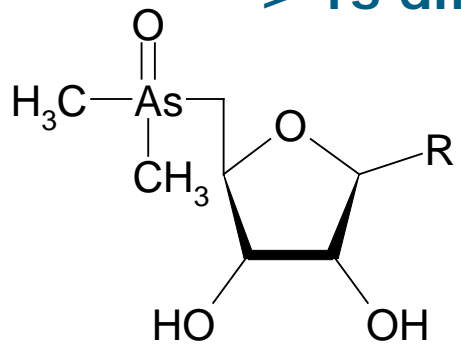
# Arsenic compounds in the marine environment



**Predominant form of arsenic in algae!!**

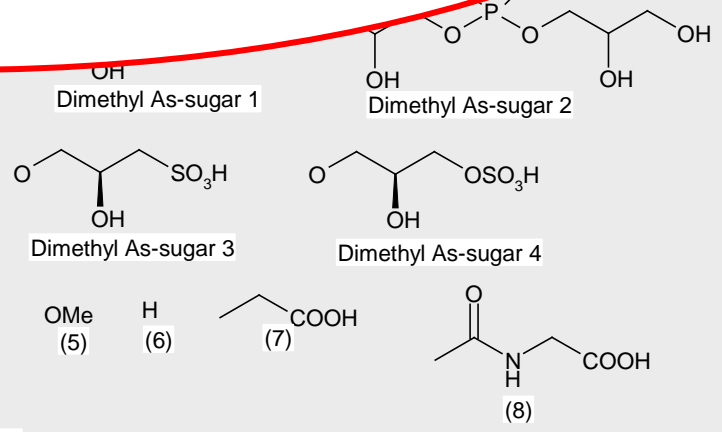
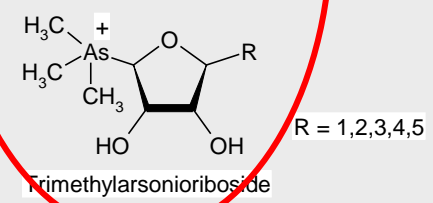
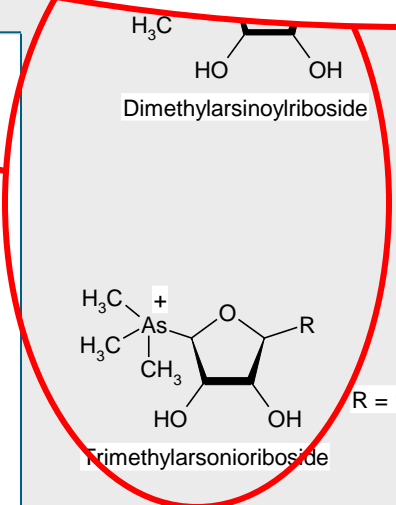
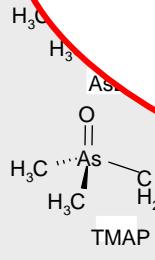
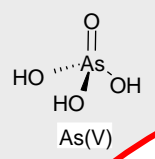
## Arsenosugars

> 15 different identified



Dimethylarsinoylribosides

Trimethylarsonioribosides



# 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:		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 lower than 2 ppm** is of particular concern in seaweed species *Hizikia fusiforme*.

**Speciation analysis is required !!**



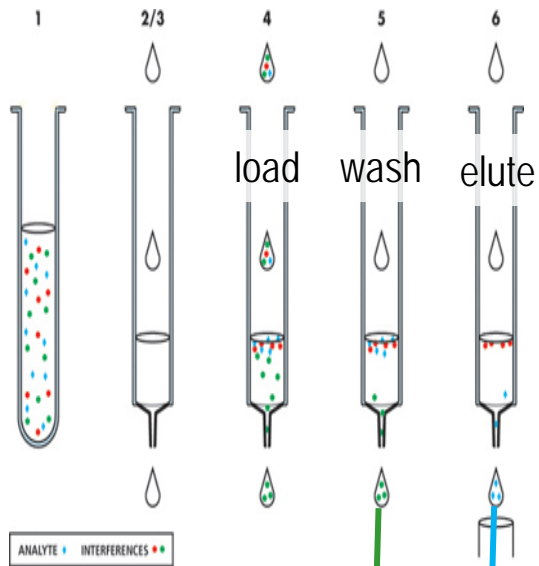
# SPE-HG-AAS – a novel speciation alternative...

$\mu$ -wave extraction

Separation by SPE

Detection by HG-AAS

Inexpensive detection system

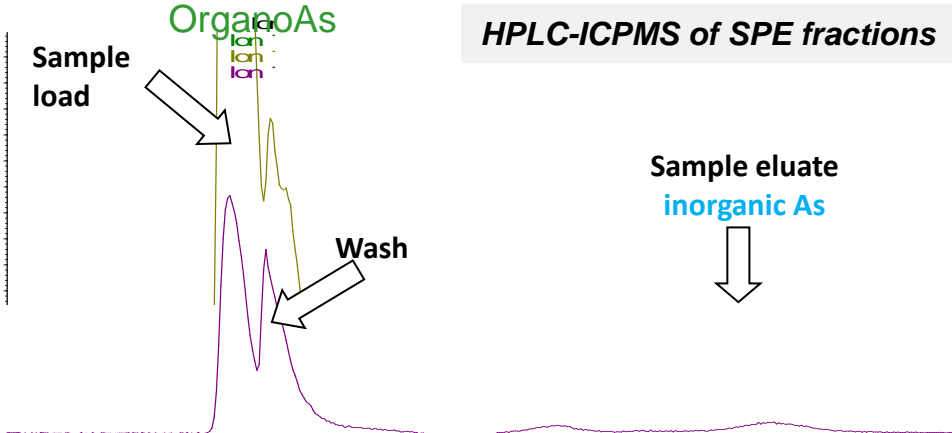
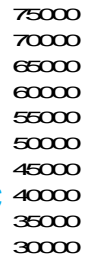


Sequential elution for selective off-line separation of inorg As from organo As species by SPE

OrganoAs compounds

Inorganic As

Abundance



# Performance characteristics from in-house validation

μ-wave extraction

Separation by SPE

Detection by HG-AAS

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

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

**A novel speciation alternative for the determination of inorganic arsenic in marine samples**

Rasmussen RR, Hedegaard RV, Herbst BK and Jens J. Sloth\*  
 DTU Food National Food Institute  
 National Food Institute, Technical University of Denmark  
 Division of Food Chemistry, Mørkøbsvej 16, DK-2860 Søborg, Denmark  
 \*corresponding author, email: [rr@food.dtu.dk](mailto:rr@food.dtu.dk)

Simple, inexpensive and fast methods for determination of the toxic inorganic arsenic species are called upon for the monitoring and control of food and feed samples. A new approach based on Microwave-Assisted Extraction (MAE) + Solid Phase Extraction (SPE) + HG-AAS has been developed, where inorganic arsenic is separated from organic arsenic species by MAE followed by determination of arsenic content by Hydride Generation (HG) Atomic Absorption Spectrometry (AAS).

**Introduction**  
 The total contents of the trace elements lead, cadmium and mercury in food and feed are regulated by EU directives (EC) Nos. 466/2001/EC, 76/2001/EC, 2002/32/EC and 2002/100/EC. However, for some heavy metals the chemical form (i.e. their speciation) is important in terms of food and feed safety. More than 50 different arsenic species have been found in the marine environment – including both soluble arsenic compounds, however it is the inorganic arsenic forms that are most toxic, whereas organoarsenic compounds are considered to have only low to intermediate toxicity. Since seafood is the major dietary source for arsenic exposure in the European population, arsenic speciation analysis of marine food and seafood commodities of great interest.

**Extraction of inorganic arsenic**  
 Several sample extraction solvents and sample preparation approaches have been tested for the extraction of inorganic arsenic. These include water, methanol, hydrochloric acid or alkaline solutions, all giving with varying results for the same reference material. Microwave assisted extraction for 30 minutes at 90 °C with 0.05 M HClO<sub>4</sub> in H<sub>2</sub>O provided the most efficient extraction of inorganic arsenic. H<sub>2</sub>O<sub>2</sub> was added to ensure quantitative conversion of arsenite As(III) to arsenate As(V) (Figure 2) and thereby facilitate the following SPE of inorganic arsenic. As(III) from organoarsenic compounds, important, no arsenic was detected in the extract.

**Figure 1: Proposal of the new SPE-HG-AAS approach for selective detection analysis of inorganic arsenic.**

**Figure 2: Chemical structures of inorganic arsenic species.**

Further details on poster and handouts



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

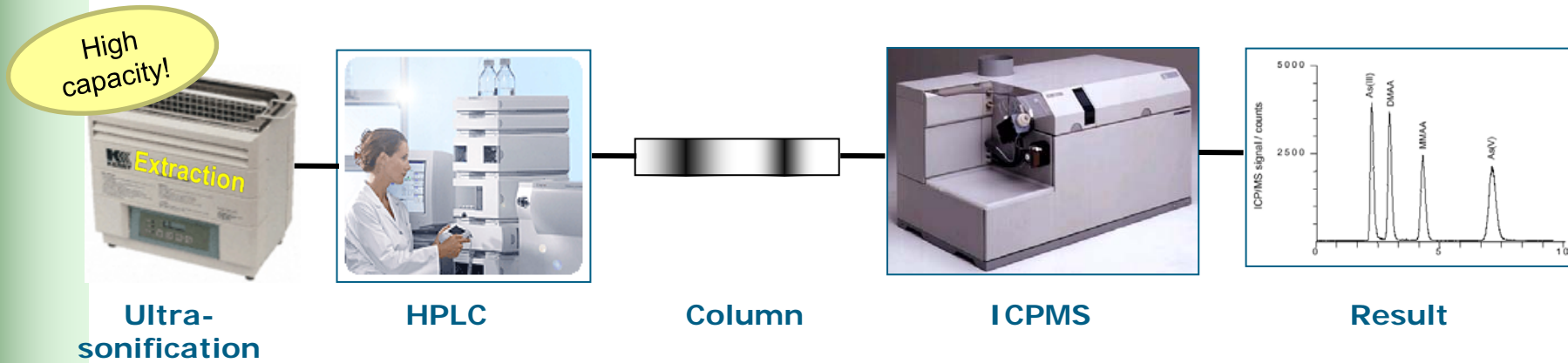
- Old PTWI value (WHO, 1988) was withdrawn
- **NEW!**  $BMDL_{1.0} = 0.3 - 8 \mu\text{g/kg bw per day}$  for inorganic arsenic
- => EU dietary exposures within this range
- => Risk to some consumers cannot be excluded
- **NEW!**  $BMDL_{0.5} = \underline{3 \mu\text{g/kg bw per day}}$  for inorganic arsenic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



- *"...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"*



# Speciation analysis of Mercury by HPLC-ICPMS

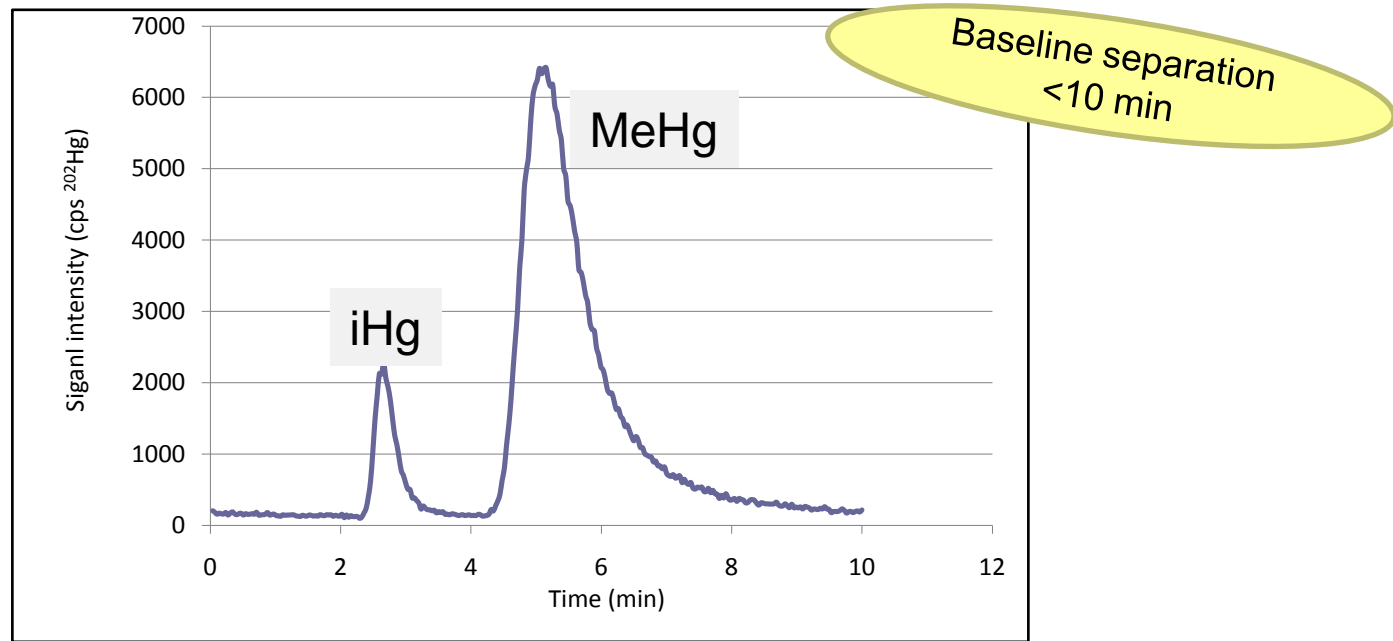


MeHg is a priority contaminant

- Adverse effects on the central nervous system
- Affects the learning ability of children
- Bioaccumulation and biomagnification in the aquatic food chain
- Biomethylation in the food chain: iHg -> MeHg
- Toxicity: MeHg > inorganic Hg
- PTWI MeHg: 1.6  $\mu\text{g}/\text{kg}$  bw/week



# Speciation analysis of Mercury by HPLC-ICPMS



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

CRM	Certified (mg/kg)	Result (mg/kg)
DORM-2 (dogfish muscle)	4.47 +/- 0.32	4.21
DORM-3 (dogfish muscle)	0.355 +/- 0.056	0.35
TORT-2 (Lobster hepatopancreas)	0.152 +/- 0.013	0.16





# Conclusion

- Total concentration info – not always sufficient!
- Speciation analysis for improved risk assessment
- Need for speciation methods for future monitoring
- ....and in feed and food control



## ***Thanks for your attention!***

Further information:

**Speciation – chemical analysis:** [jjsl@food.dtu.dk](mailto:jjsl@food.dtu.dk) (Jens J. Sloth)



Miscellaneous info on speciation: EVISA homepage; [www.speciation.net](http://www.speciation.net)  **evisa.**

Further developments in **CONFIDENCE**: [www.confidence.eu](http://www.confidence.eu)

