National Food Institute

Technical University of Denmark

DTU

Heavy metal speciation: why and how ?

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National Food Institute (DTU Food)

Technical University of Denmark

Today's agenda

- The National Food Institute
- Definition of speciation
- Toxicity and speciation
- Speciation and legislation (EFSA)
- Analytical methodologies for speciation
- Examples of speciation
- Projects:
  - CEN standard
  - CONffIDENCE
- Developing of new methods speciation (HPLC-ICPMS and SPE)
- Summary and Conclusion

- **Total of 60 employees**
- **Externally funded research projects**
- **Research-based advise to authorities**
- **National reference lab for food analysis**
- **Mass spectrometry!!**

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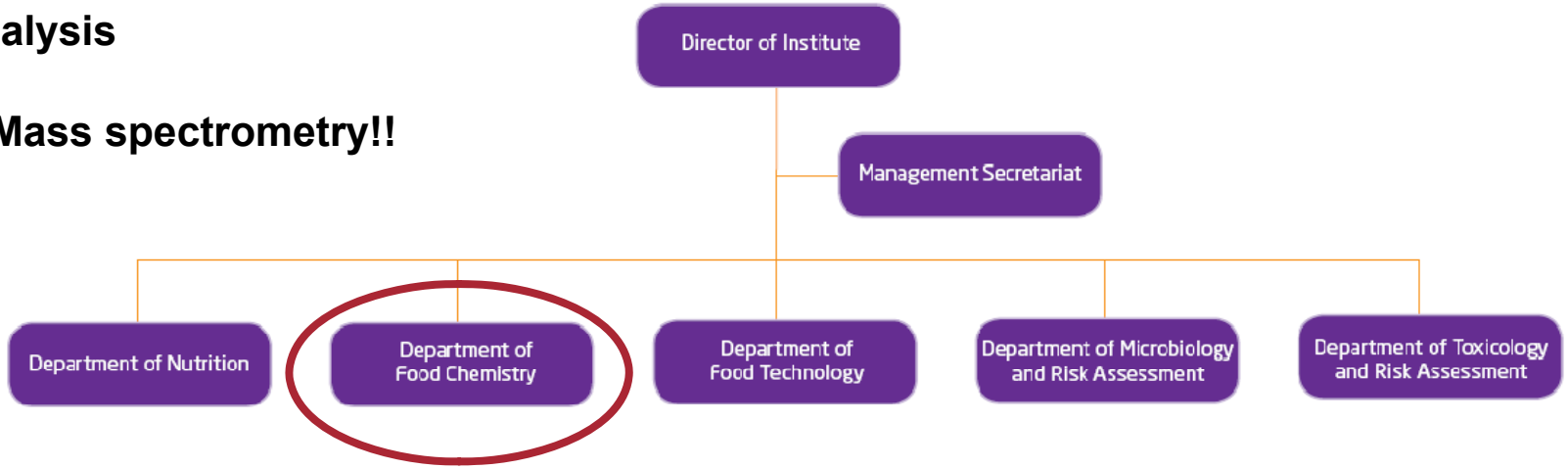


Pesticides
Vet drug residues
Biotoxins
Bioactive compounds
POPs
Vitamins
Trace elements

Speciation - definition

(IUPAC definition: Templeton et al., Pure and Appl. Chem., 2000, 72, 143)

Chemical species:

- specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.

Speciation of an element:

- distribution of an element amongst defined chemical species in a system.

Speciation analysis:

- analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample.

Speciation – some historical milestones

- **"Fish arsenic"** (Chapman 1926)
  "...a different (and non-toxic) compound than As_2O_3"

- **Minamata, mercury,** Japan (1950'ies)
  $MeHg^+$ emissions from industry into fish ➡ man

NIMD

- **Archachon Bay, TBT,** France (1980'ies)
  TBT from antifouling agents -> oysters

- **EU Tematic network "speciation 21"** (1997-2000)
  Participants from research, industry og authorities

evisa.

- **EVISA** (European Virtual Institute for Speciation Analysis)(2003)
  Information and technology transfer in the framework of speciation
  See more info at the EVISA homepage: www.speciation.net

Pollutants in formulated feed

Fish meal

Heavy metals such as
Mercury
Arsenic

Fish oil

Dioxins
Dioxin-like PCBs
ated flame retardant
(Persistent Organic Pollutants)

Effect on Food safety

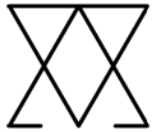
Vegetable meal

GM ingredients
(Gene Modified)
Metals e.g. cadmium

Vegetable oils

Pesticides

Arsenic - occurrence



Arsenic alchemical symbol

High concentrations of arsenic has been found in samples from the marine environment.

Seawater	1	-	2	µg/L
Marine fish	0,2	-	**>100**	mg/kg
Marine invertebrates	0,2	-	>**100**	mg/kg
Marine algae	0,02	-	40	mg/kg
Freshwater fish	<0,01	-	2	mg/kg
Terrestrial biota		<0,2		mg/kg

All results on wet weight basis

Marine organisms can bioaccumulate arsenic by a factor of up to 100.000 compared with seawater!!!

Analysis of total content vs. speciation analysis

Analysis of ~~total concentration~~

- **Arsenic**
- **Tin**
- **Mercury**
- **Lead**

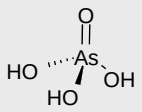
Speciation analysis is required!!

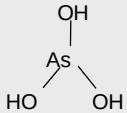
Aim: get info

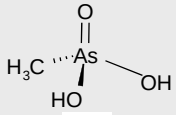
- mobility
- bioavailability
- lifetime, fate and metabolism
- chemical and physical activity

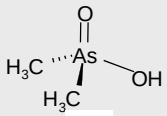
these characteristics are **species** related

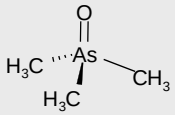
Arsenic species in the marine environment

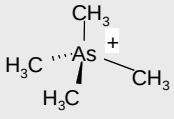

As(V)


As(III)


MA


DMA


TMAO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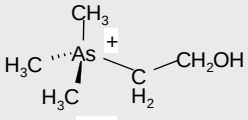
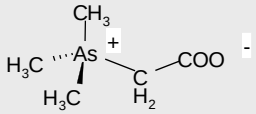
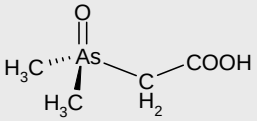
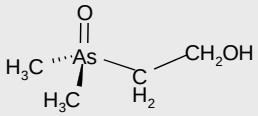
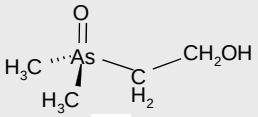

AC


AB


DMAA


DMAE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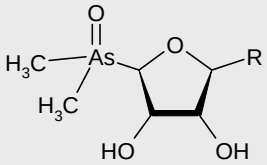
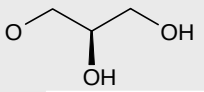
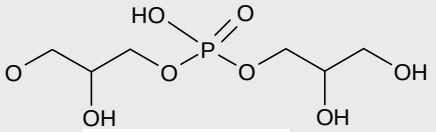
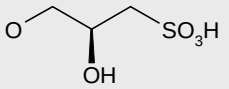
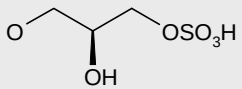
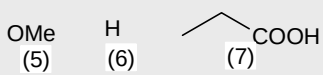

Dimethylarsinoylriboside

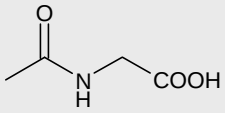

Dimethyl As-sugar 1

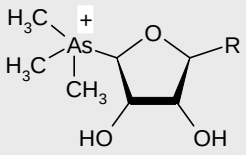

Dimethyl As-sugar 2


Dimethyl As-sugar 3


Dimethyl As-sugar 4


OMe (5) H (6) COOH (7)


(8)


Trimethylarsonioriboside

R = 1,2,3,4,5

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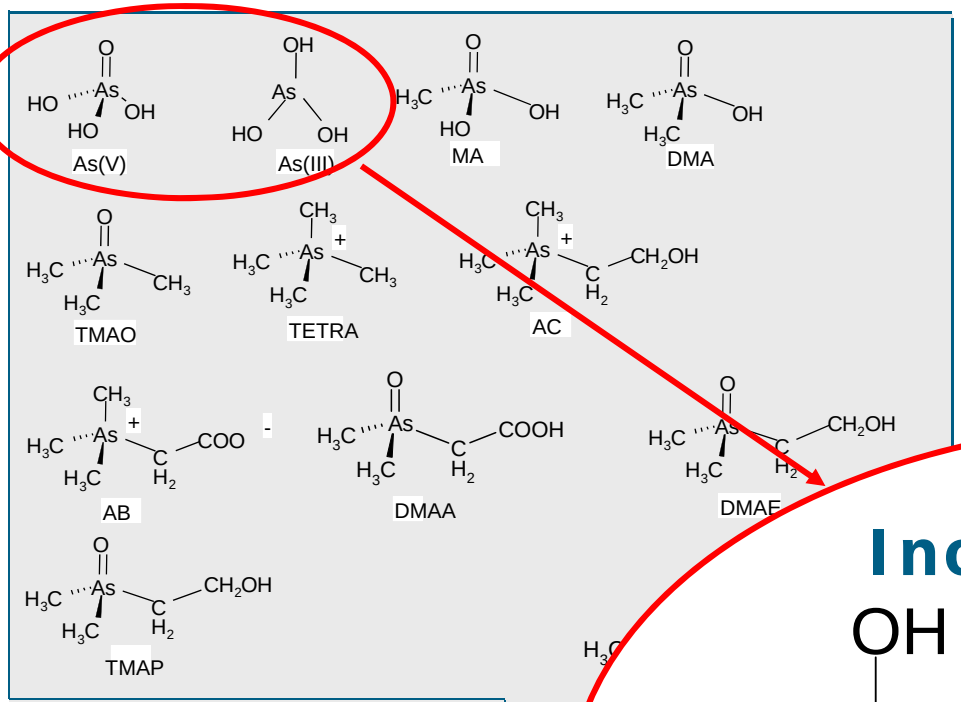
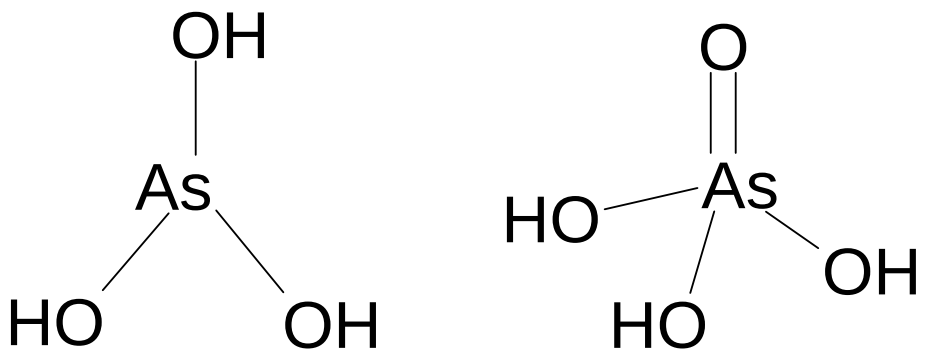
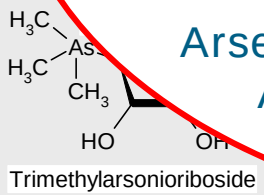
Arsenic compounds in the marine environment



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

Inorganic arsenic





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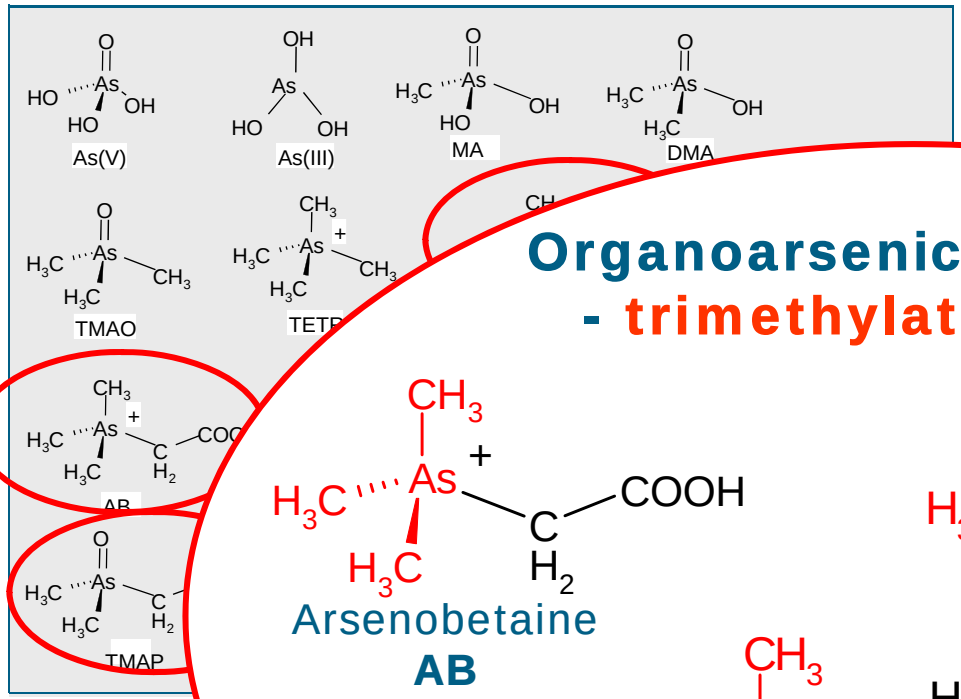
Arsenic compounds in the marine environment

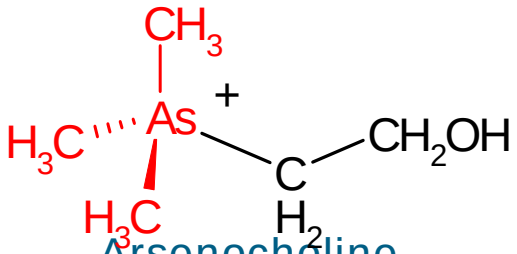


More than **50** different arsenic species have been found in the marine

Organoarsenic compounds - trimethylated species

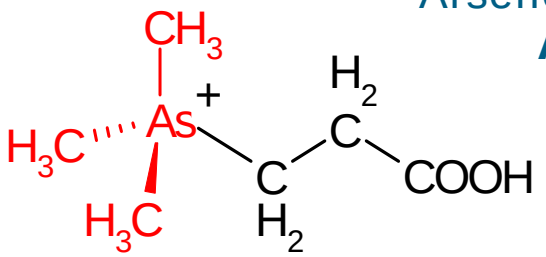
Arsenobetaine

AB



Arsenocholine

AC



Trimethylarsoniopropionic acid

TMAP

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Arsenic - toxicity

Toxicity: **As(III)** > **As(V)** > TETRA > MA > DMA > AC/AB

Inorganic arsenic

LD_{50} - values (mg/kg)	
As(III)	**15-42**
As(V)	**20-800**
TETRA	890
MA	700-1800
DMA	1200-2600
AC	6500
AB	>10000

Values for mice and rats

Kaise & Fukui (1992); Shiomi (1994); Donohue & Abernathy (1999)

Arsenic – chronic toxicity

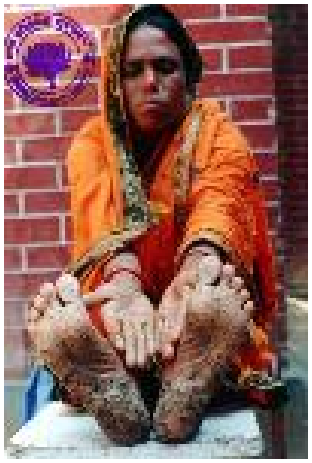
Long term exposure => skin diseases

- Keratosis, gangrene, melatosis
- Skin cancer

… and also

- lung, kidney, liver, bladder cancers
- Cancer slope factor: 1.5 (mg kg^{-1} $day^{-1})^{-1}$

  (US EPA 2005)



WHO PTWI for ***inorganic arsenic***: 15 µg/kg bw/week

(**P**rovisional **T**olerable **W**eekly **I**ntake)

For a 70 kg person => 150 µg / day

Commission Directive 2003/100/EC on animal feed

Max levels for total arsenic

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 (8)	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 (9)
	— phosphates and calcareous marine algae	10
	— calcium carbonate	15
	— magnesium oxide	20
	— feedingstuffs obtained from the processing of fish or other marine animals	15 (9)
	— seaweed meal and feed materials derived from seaweed	40 (9)

Footnote in the Commission directive

mineral feedingstuffs	12

(9) 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. This analysis is of particular importance for the seaweed species *Hizikia fusiforme*.'

Total arsenic in fish feed products

Sloth et al, Aquaculture Nutr., 2005, 11, 61-66

33% > ML

Product	N	Median	Mean	SD	Range
Complete feedingstuffs	39	5.87	5.80	1.17	3.40 - 8.34
Fish meal	10	7.93	7.70	4.00	3.62 - 18.2
Fish oils	6	11.30	11.17	1.8	

10% > ML

Results in mg kg^{-1}

ML = 15 mg As / kg

Marine feedingstuffs

Complete feedingstuffs

Inorganic arsenic in fish feed products

Sloth et al, Aquaculture Nutr., 2005, 11, 61-66

Product	N	Range	% of total As
Complete Feedingstuffs	13	10 - 61	0.18 - 1.20
Fish meal	10	All < 7	-

Results in µg kg^{-1}

Total arsenic in fish feed products

Sloth et al, Aquaculture Nutr., 2005, 11, 61-66

Product	N	Median	Mean	SD	Range
Complete Feedingstuffs	39	5.87	5.80	1.17	3.40 - 8.34
Fish meal	10	7.93	7.70	4.00	3.62 - 18.2
Fish oils	6	11.30	11.17	1.81	9.11 – 13.51

1/3 of arsenic in the feed is lipid-bound!

Results in mg

Sloth et al, J.Agric.Food Chem., 2005, 53, 6011-6018

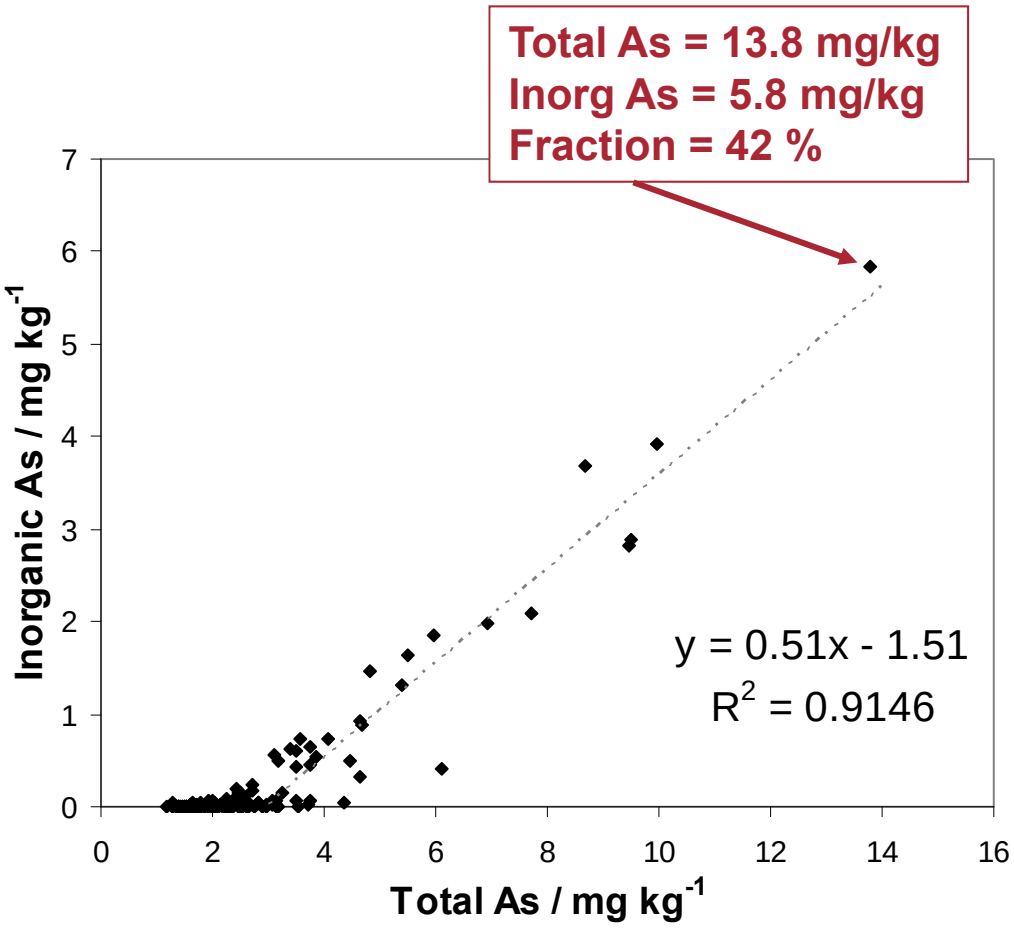
Sample identification	Inorganic arsenic	Total arsenic
Salmon (*Salmo salar*)	< 0.0006	1.9 ± 0.2
Cod (*Gadus morhua*)	< 0.0006	17 ± 2
Cod (*Gadus morhua*)	< 0.0006	15 ± 2
Wolffish (*Anarhichas lupus*)	< 0.0006	4.1 ± 0.5
Wolffish (*Anarhichas lupus*)	< 0.0006	31 ± 4
Anglerfish (*Lophius piscatorius*)	< 0.0006	15 ± 2
Anglerfish (*Lophius piscatorius*)	< 0.0006	44 ± 6
Atlantic halibut (*Hippoglossus hippoglossus*)	< 0.0006	12 ± 1
Mackerel (*Scomber scombrus*)	< 0.0006	1.7 ± 0.2
Mackerel (*Scomber scombrus*)	< 0.0006	2.8 ± 0.4
Herring (*Clupea harengus*)	< 0.0006	1.5 ± 0.2
Herring (*Clupea harengus*)	< 0.0006	1.7 ± 0.2
Herring (*Clupea harengus*)	< 0.0006	1.7 ± 0.2
Tuna fish (Thunnus alalunga)	0.008 ± 0.001	0.9 ± 0.1
Lobster, tail meat (*Homarus gammarus*)	0.0006	14 ± 2
Lobster, head and thorax meat (*Homarus gammarus*)	0.057 ± 0.005	22 ± 3
Crab, white meat (*Cancer pagurus*)	0.026 ± 0.002	32 ± 4
Crab, head and thorax meat (*Cancer pagurus*)	0.060 ± 0.009	26 ± 3
King crab, white meat (*Paralithodes camschaticus*)	0.005 ± 0.001	26 ± 3
Norway lobster (*Nephrops norvegicus*)	0.020 ± 0.003	23 ± 3
Shrimp (*Pandalus borealis*)	< 0.0006	3.8 ± 0.5
Shrimp (*Pandalus borealis*)	< 0.0006	60 ± 8
Shrimp (*Pandalus borealis*)	< 0.0006	67 ± 8
Horse mussel (*Modilous modiolus*)	0.0012 ± 0.002	3.4 ± 0.4
Scallop muscle (*Pecten maximus*)	0.008 ± 0.001	3.1 ± 0.3
Oyster (*Ostrea edulis*)	0.014 ± 0.002	1.8 ± 0.2
Mink whale (*Balaenoptera acutorostrata*)	< 0.0006	0.61 ± 0.08
Harp seal (*Pagophilus groenlandicus*)	< 0.0006	0.9 ± 0.1
Hooded seal (*Cystophora cristata*)	< 0.0006	0.22 ±0.03

Fish muscle

Crustaceans & bivalves

Marine mammals

For all samples inorganic arsenic constitutes less than 1% of total arsenic

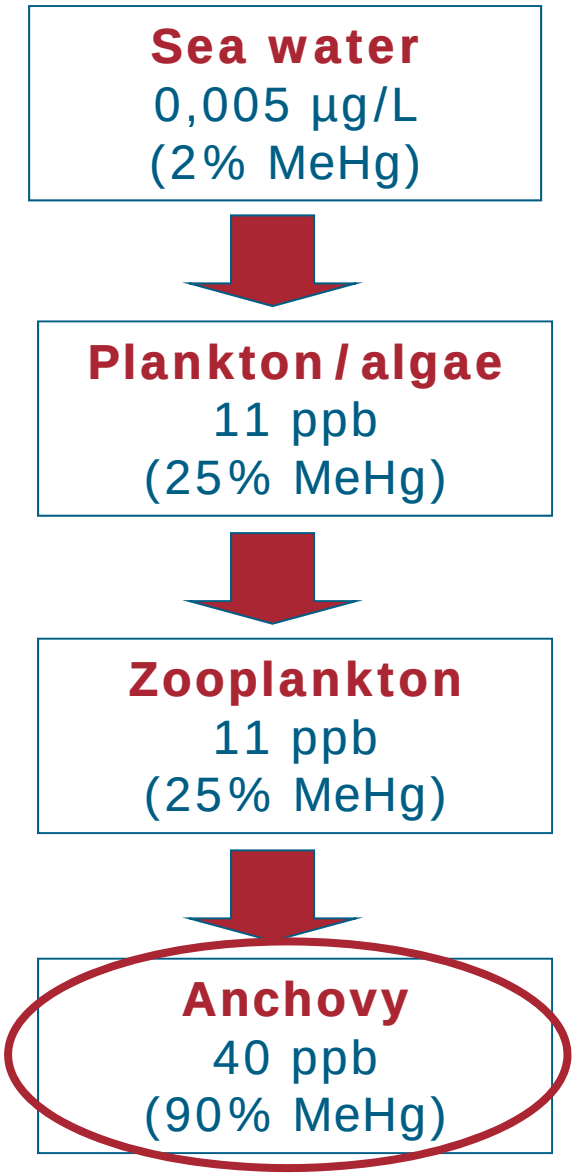


Data from 175 blue mussel (*Mytilus edulis*) samples collected along the Norwegian Coastline in 2004-06.

Sloth and Julshamn 2008, J. Agri.Food Chem.

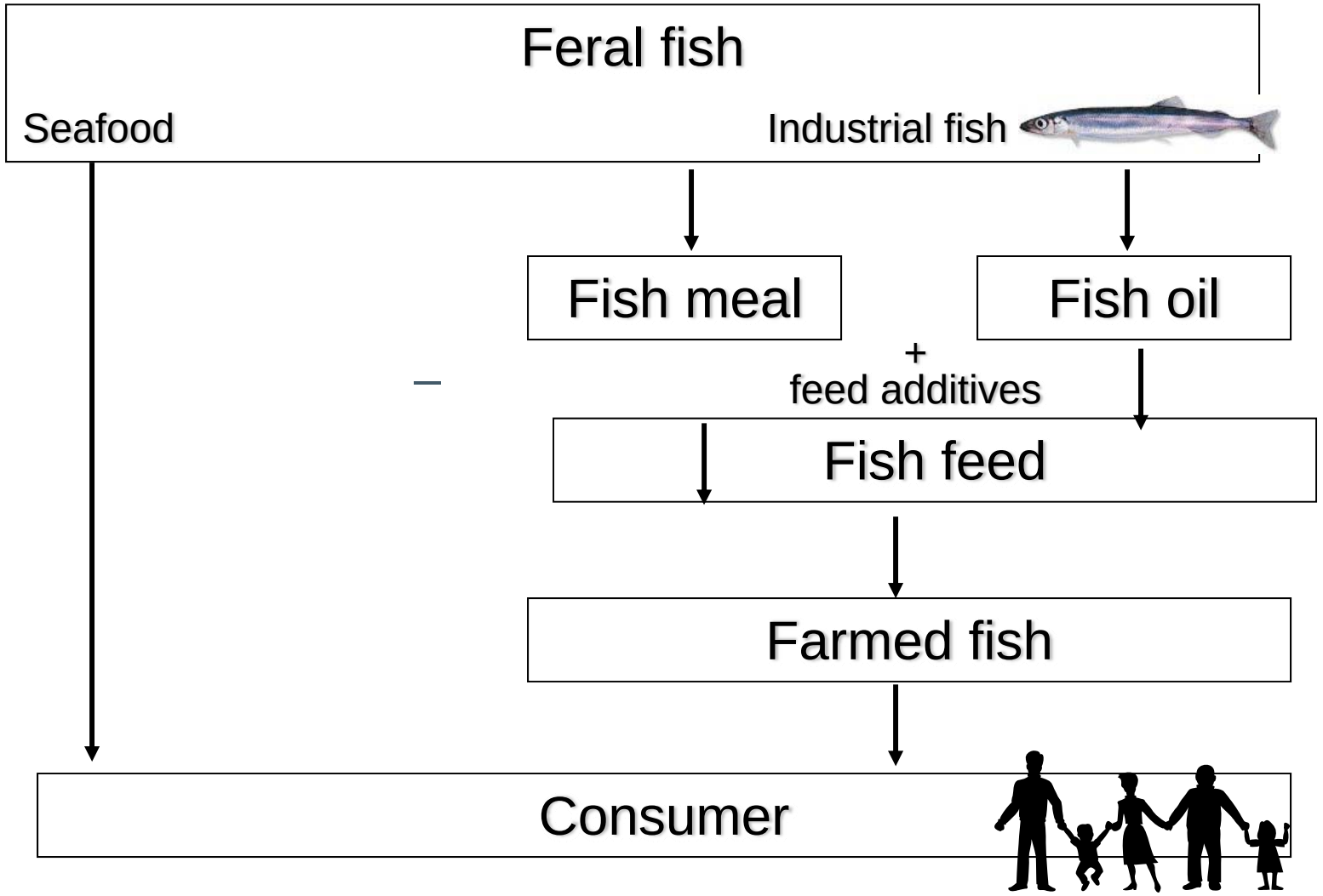
Mercury speciation

- Effects the central nerve system
- Learning ability for children
- Bioaccumulation in the food chain
- Toxicity: MeHg > inorganic Hg
- Biomethylation: Inorganic Hg -> MeHg

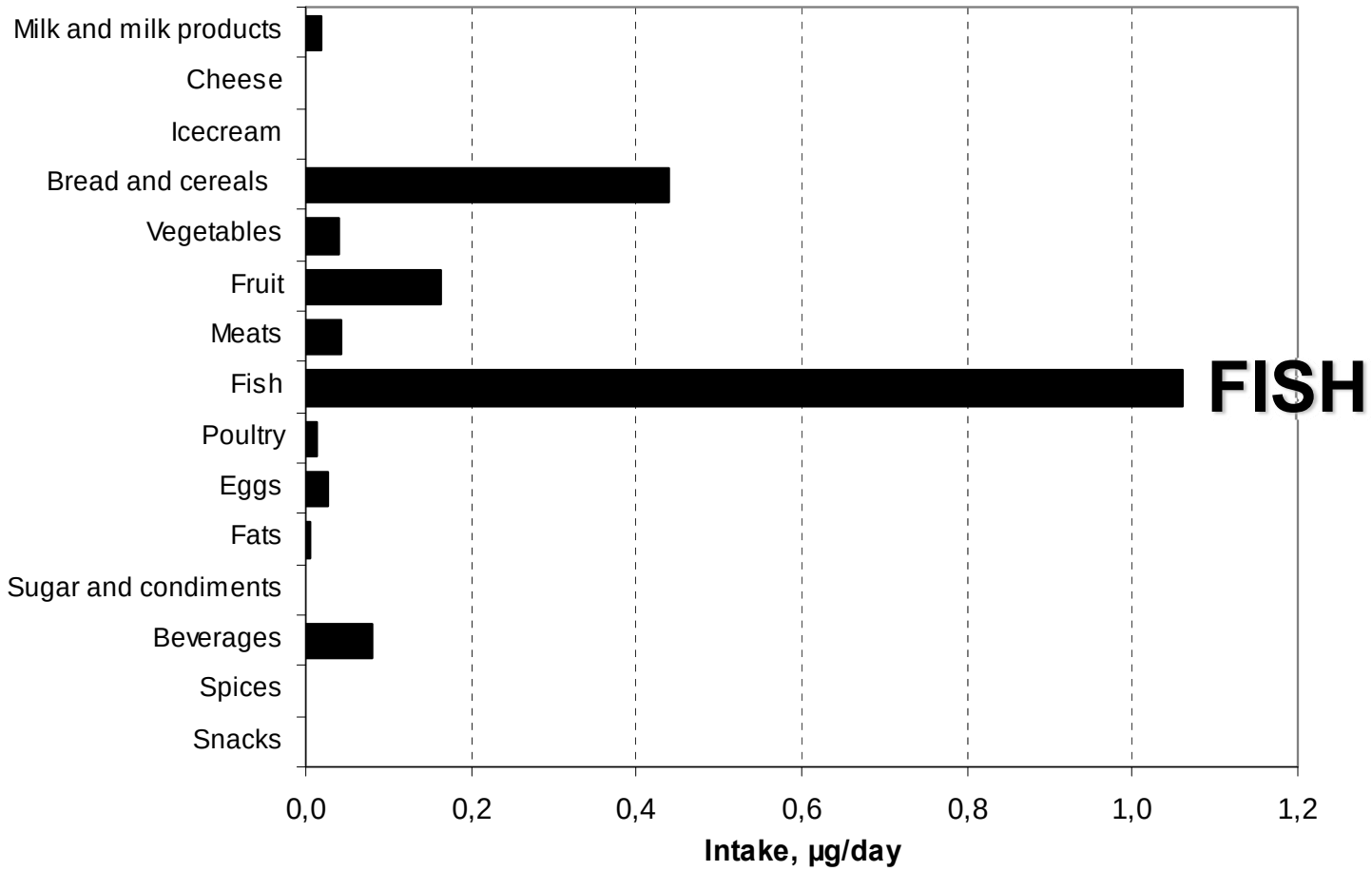


Ref: Bjerregaard, 1988

Mercury accumulates in food chain



Mercury intake from food



Data fra FVST/DFVF's overvågningssystem

Directive No (EC) 2002/32 of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.

4. Mercury

Feed materials

-feedingstuffs of fish/marine animals

0.1 **mg/kg**

0.5 **mg/kg**

th

Complete feedingstuffs with the exception of:	
— complete feedingstuffs for dogs and cats	0,4
Complementary feedingstuffs except:	0,2
— complementary feedingstuffs for dogs and cats	

Scientific Committee on Animal Nutrition (SCAN) in 2003 concluded that a more detailed risk assessment of the presence of mercury in animal feed and the possible effects for animal health and public health is necessary and that this detailed assessment should address the risks related to the organic forms of mercury.

- PTWI = 5 µg / kg bodyweight (Total Hg)
- PTWI = 1.6 µg/kg bodyweight (MeHg)

There is a need for methods for future monitoring and control with heavy metals speciation for feed

The demand is:

- Simplification of existing method

- Inexpensive instrumentation
- Semi-automated

- Robust
- Easy-to-use

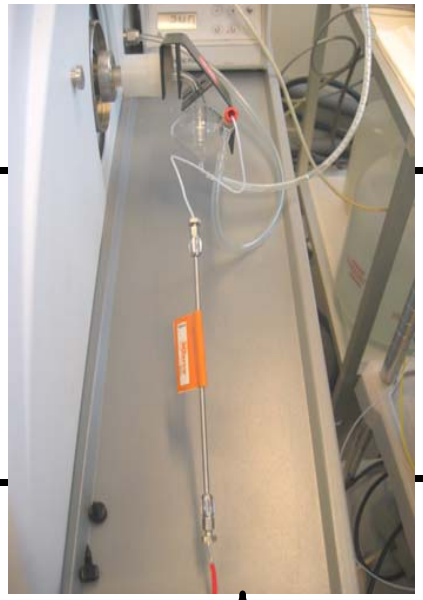
- Applicable in routine control laboratories

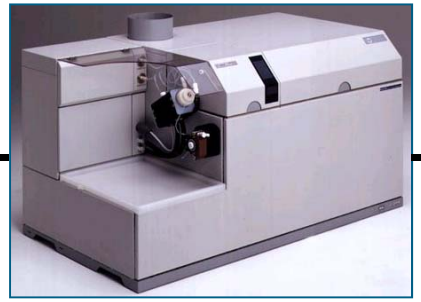
Speciation analysis

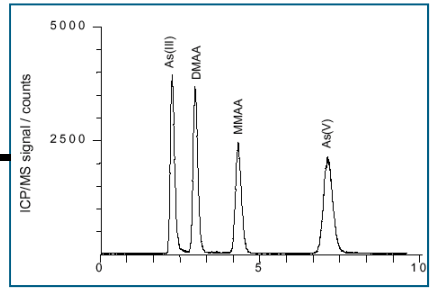
- **Online separation**: **HPLC ICP-MS**
- **Off line separation**: **SPE-AAS**
- **Screening methods**: **Biosensors**



HPLC-ICPMS – the speciation workhorse







HPLC

Sample introduction

ICPMS

Element specific detection

Result

Chromatogram

- Simple coupling from column outlet to ICPMS
- HPLC flow rate 0,2 - 2 ml/min matches ICP-MS' demands

Analysis of inorganic arsenic by SPE-HGAAS

Microwave assisted hydrolysis:

Freeze drying of sample
(Addition of solvent)

↓

Microwave treatment 20 min, 90°C in slightly acetic conditions and H_2O_2

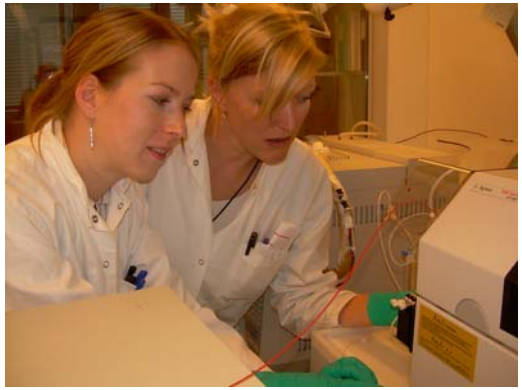
I: Solubilisation of sample matrix

II:Conversion of As(III) TO As(V) by H_2O_2

III: Insure no degradation of other arsenic species to As(V)

↓

Adjust pH of sample to pH 6



Extraction of inorganic arsenic by SPE

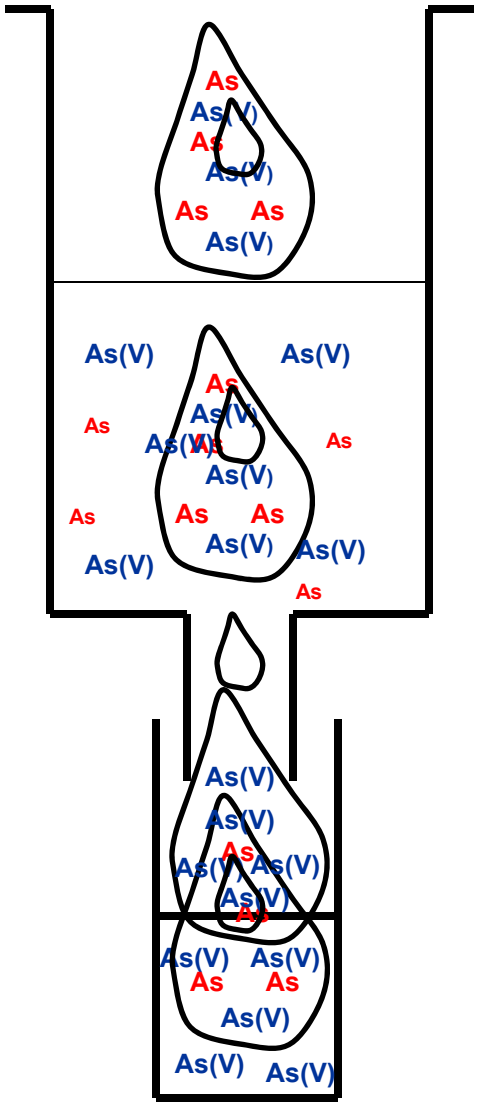
SAX (strong anionic exchange)

1. Wash of the column with methanol
2. Condition the cartridges with solvent

3. Addition of sample. Inorganic arsenic in the form of As(V) is retained on the cartridges

4. Elute interferences from other arsenic species with acetic acid

5. Elution of inorganic arsenic as As(V) with HCl

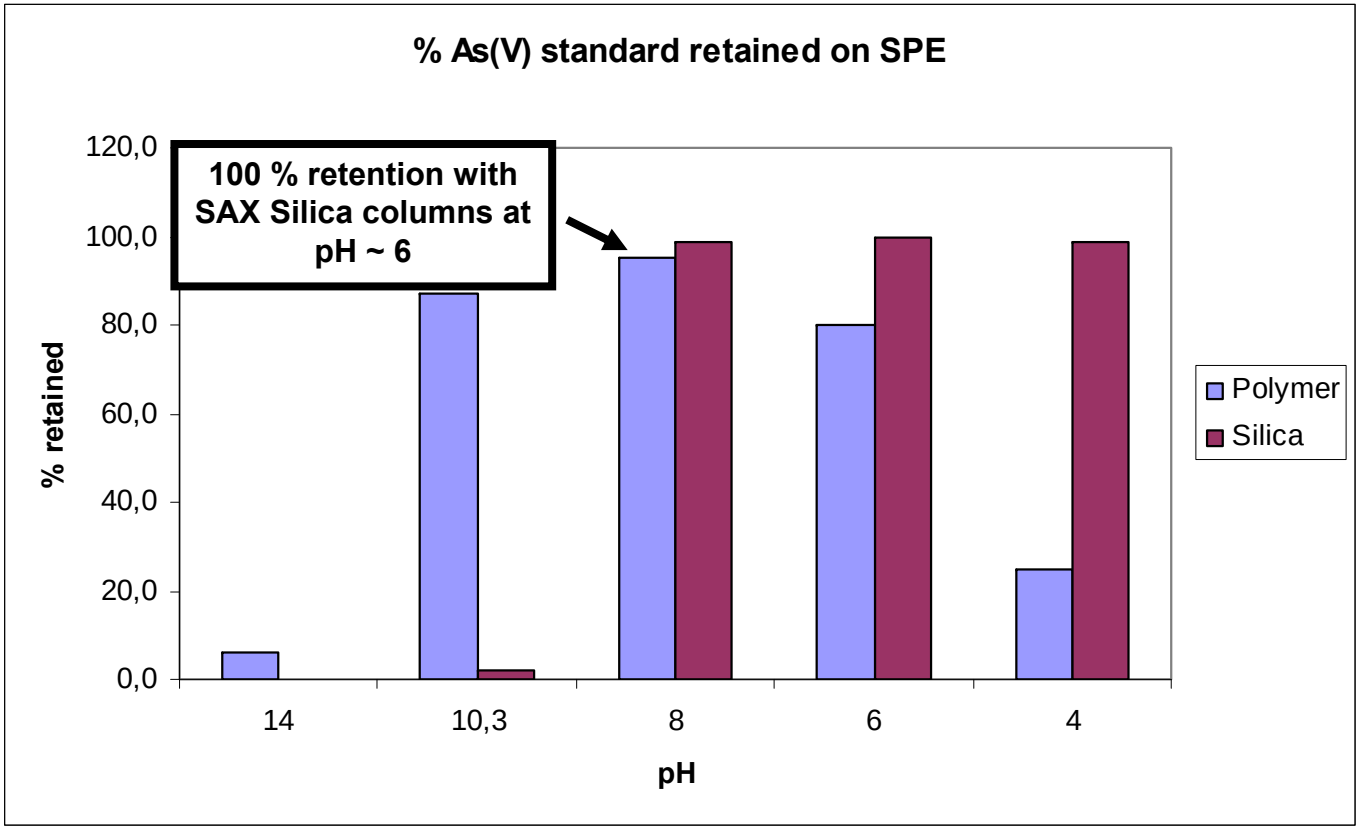


As ~ Other arsenic compounds

National Food Institute

DTU

Solid phase extraction (SPE): Silica versus polymer



SPE column important only about 80 % retention on polymer based

PRE-REDUCTION AND DETECTION

- Electrically heated cell
- Prereduced to As(III)

AAS compared to ICP-MS

Preliminary Results- not published

	AAS (ppm)	ICP (ppm)
Tort-2 (Lobster hepatopancreas)	0,94	0,95
Blue mussel	0,38	0,37
Shrimp	0,22	0,20

- Detection limits AAS: below 0.2 ppm
- Ongoing test of several different marine matrices
- In-house validation
- Collaborative trial with 5-6 laboratories participation early 2010
- Towards a European standard on 2011

Summary

- Total concentration of trace elements does not always provide adequate information on bioavailability and toxicity
- Speciation analysis is required
- HPLC- and GC-ICPMS are versatile tools for trace element speciation analysis
- **Arsenic speciation analysis by HPLC-ICPMS**

  - inorganic arsenic is the most toxic form

  -**Alternatively by SPE-HG-AAS**
- **Mercury speciation analysis by GC-ICPMS/HCLP-ICPMS**

  - seafood are potential sources of exposure

  - **Alternatively by SPE-HG-AAS**

Thanks for your attention!!!