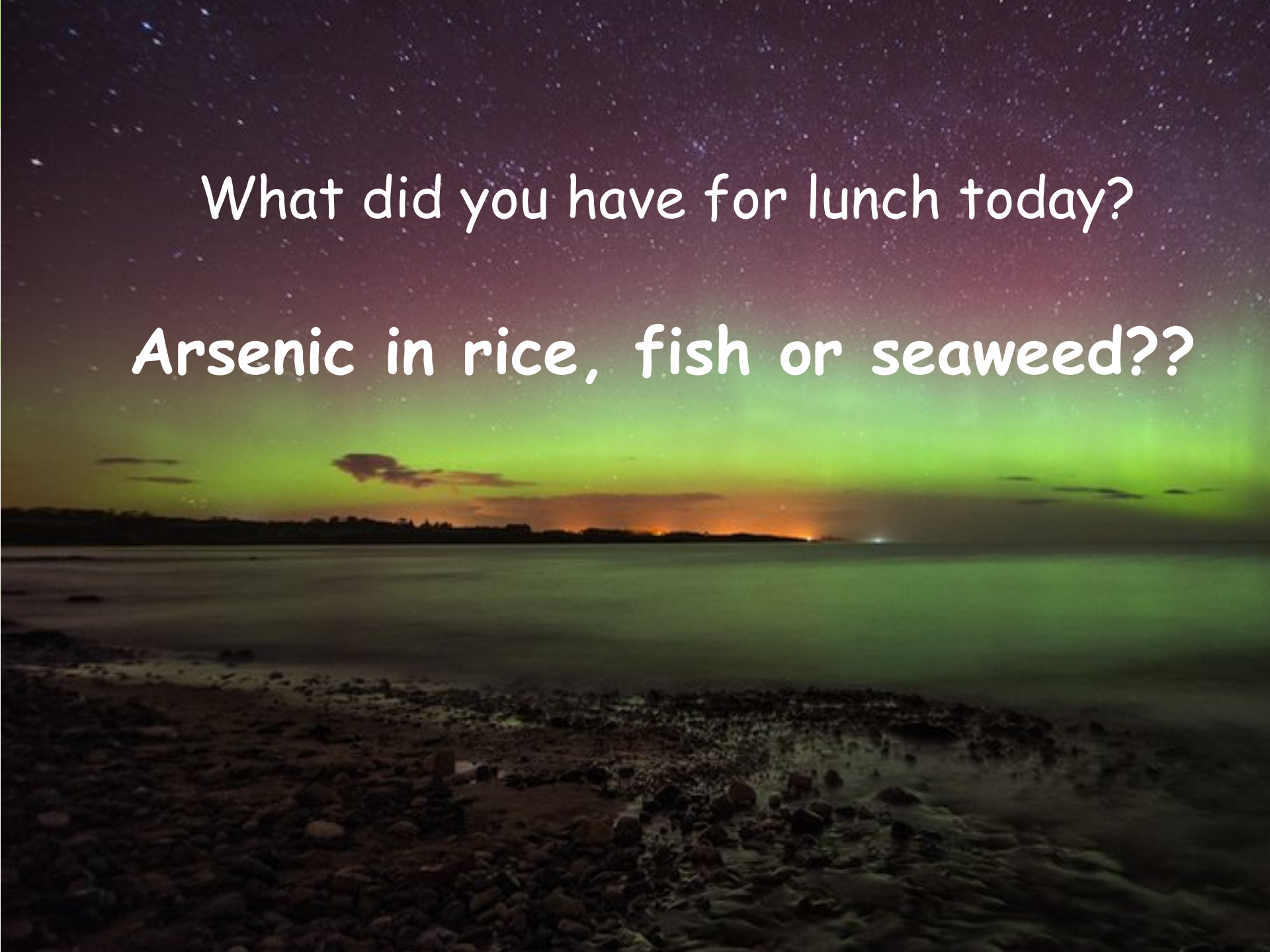
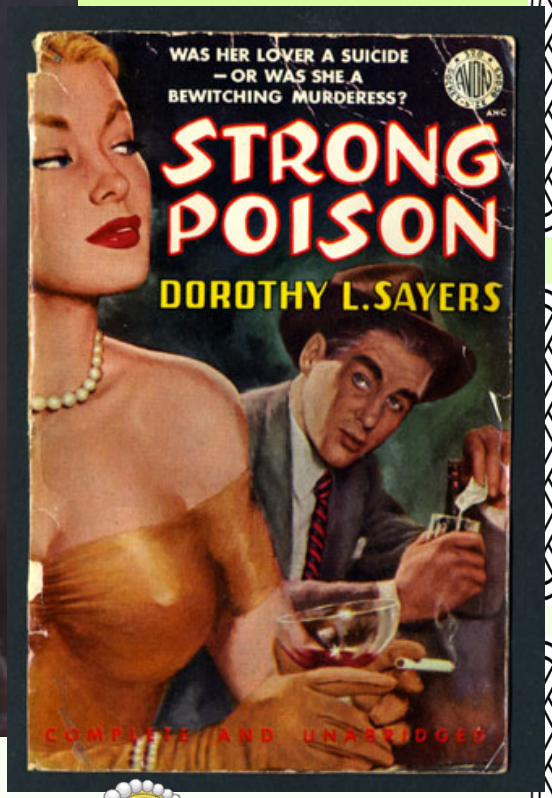

What did you have for lunch today?
Arsenic in rice, fish or seaweed??

Arsenic – a metaphor for a poison



Use of As in antiquity,
e.g. as "succession powder"

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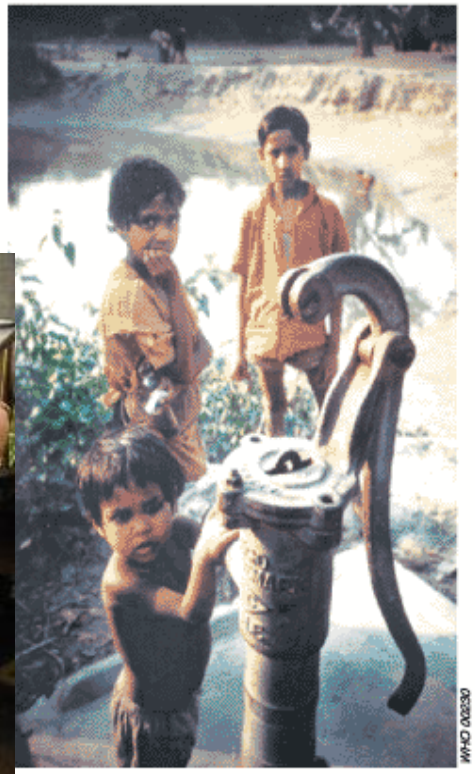
More than 60 Million people are exposed to elevated levels of Arsenic in their drinking water..

Fig. 1. **Skin lesions due to arsenic poisoning**

Chandra Banu (Feet)
AN ARSENICOSIS PATIENT (Before)

Fig. 2. **Children near a tube-well disconnected due to contamination of water with arsenic**



WHO level for As in drinking water: < 10 µg/L
Max. daily intake: < 10 µg !!!

The Arsenic problem in South East Asia

1970 problem with water-born infectious diseases

BGS/Unicef provides access to uncontaminated water (e.g., 11 Mio boreholes in Bangladesh)

Mid 80s discovery of As in groundwater (2 mg/L), up to 200 times the WHO guideline !

Reasons still debated (e.g.)

- Arsenopyrite oxidation
- Reduction of (Fe(O(OH)) liberates As

People suffer from

- Keratosis
- Highly elevated cancer rate (skin, lung, bladder)

Arsenic: class I carcinogen

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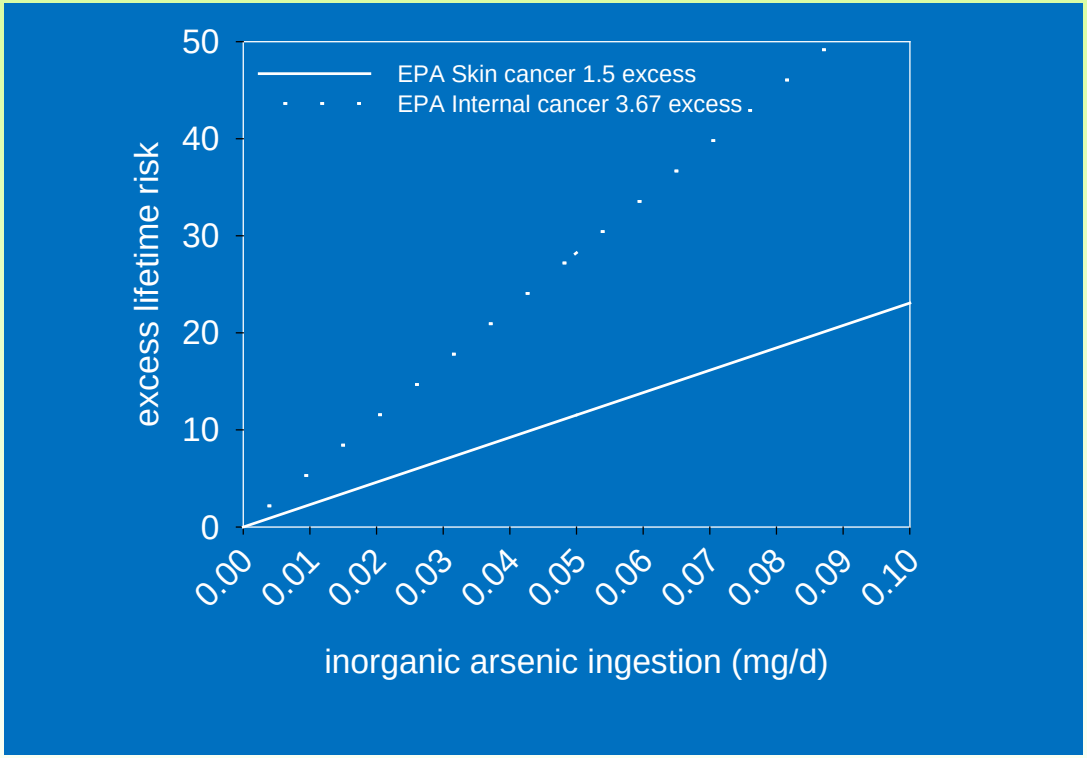
TOXICOLOGICAL IMPLICATIONS:
EPA cancer slopes excess lifetime risk per 10,000:



→US EPA target for any given source should not exceed a cancer risk of 1 in 10,000 !

Excess lifetime risk per 10,000 based on consumption of 1 L water @ 10 µg/L per day, or a total of 10 µg As / day.

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Arsenic is not only a problem in drinking water!

0.001

0.05-0.09

0.005-0.01

0.22-1.1

0.5-1,5

3.0

>500

20-200

0.01 mg/kg

0.1 mg/kg

1 mg/kg

Arsenic

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Arsenic in irrigation water in Bangladesh

Irrigation gives two harvests, but…
there is As in Bangladesh Rice!

PN Williams et al. Environ. Sci. Technol. 2006, 2007

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Arsenic Speciation and their toxic potential

UK guideline since 1959: Regulation of arsenic in food:

*… **any food other than fish, edible seaweed***

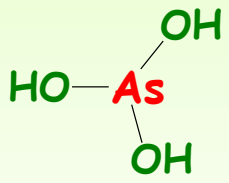
may not contain … ceeding 1 mg/kg.

→ Arsenic in fish … mless !!!

H_3C—

OH

H



Arsenobetaine is … **…organic Arsenic**

inert and is the major compound in fish and seafood

Class I carcinogen

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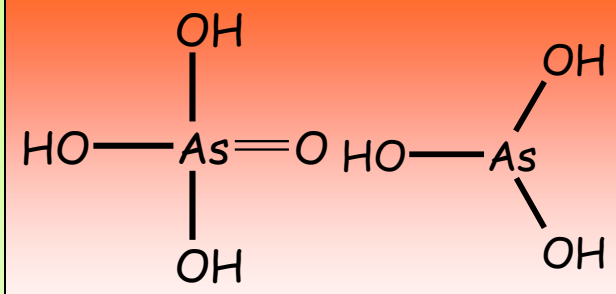
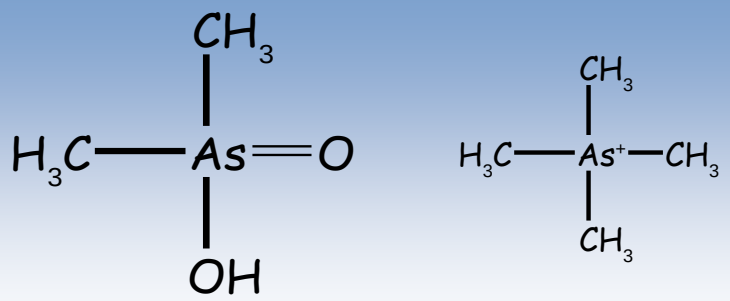
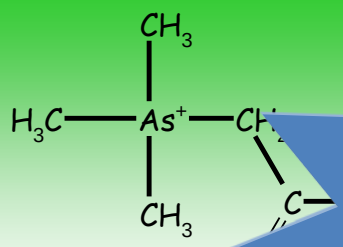
Classes of arsenic species in food commodities



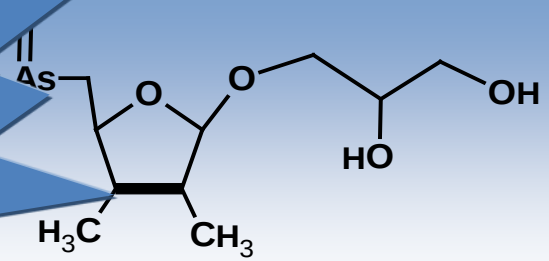
Inorganic As (iAs)



Methylated arsenic (DMA, Tetra)

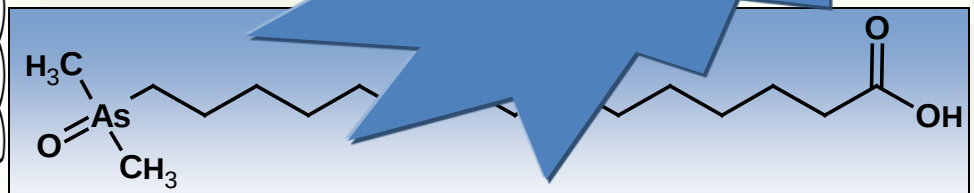


Arsenobetain

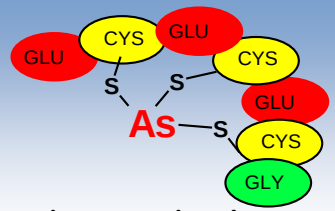


nosugars (AsSugar-OH, -SO_4.)

More than 100 naturally occurring Arsenic species



Arsenolipids (AsHC, AsPL, AsFA)



Arseno-phytochelatins (AsPC3)

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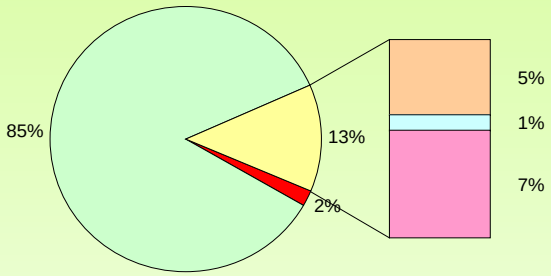
J. Feldmann & EM Krupp: Anal Bioanal Chem (2011), **399**, 1735-1741.

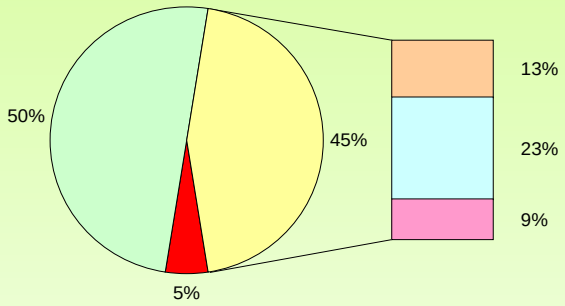
Food control: Simplification of Speciation analysis by determining only three fractions of arsenic

Fish

1 mg/kg



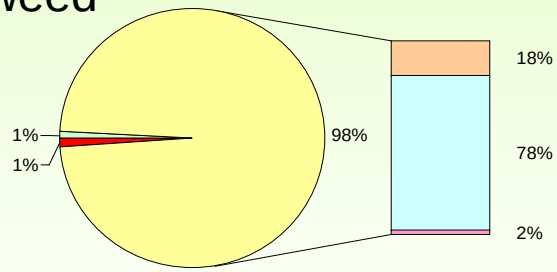
Molluscs



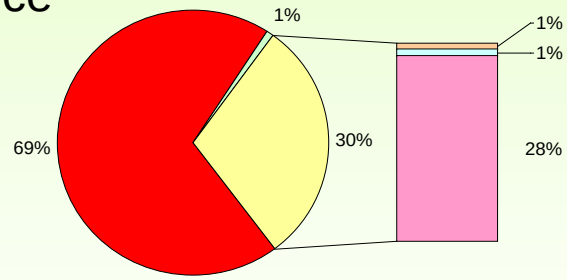
3 mg/kg

Seaweed

100 mg/kg



Rice



0.3 mg/kg

toxic (iAs)

non-toxic (AsB)

potentially toxic

arsenic fractions

fat-soluble As (e.g. As-Lipids)

arsenosugars

other organoarsenicals

potentially toxic

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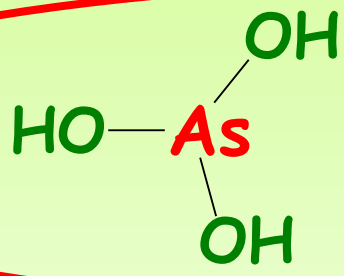
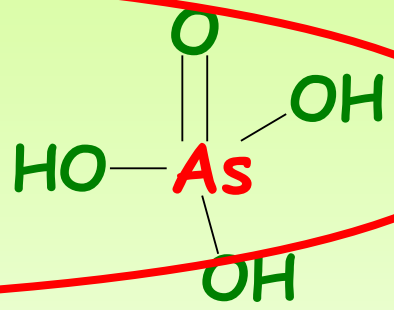
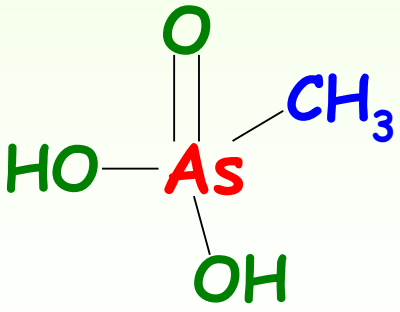
As-speciation in rice grain: simple

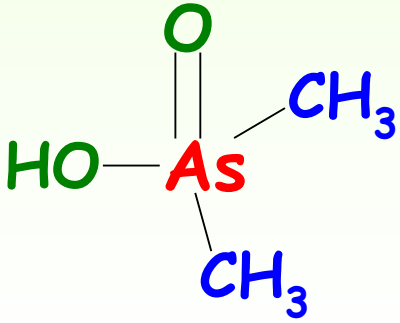
"inorganic As"

As(III) 

As(V) 

"organic As"

MA(V) 

DMA(V) 

TETRA $(CH_3)_4As^+$

HR Hansen et al.
J. Environm. Monit. (2011)

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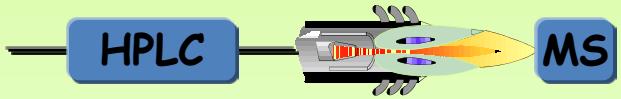
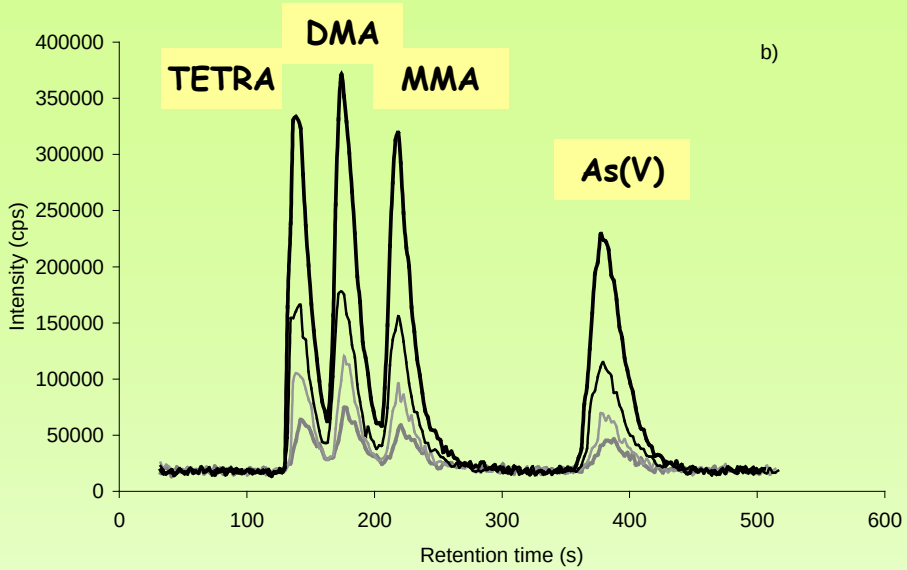
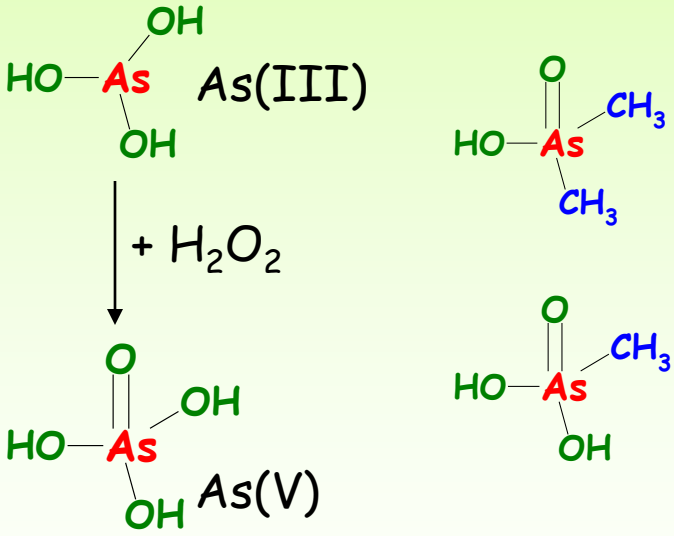
Inorganic As (iAs) in rice is (relatively) simple by using HPLC-ICPMS







HR Hansen et al, JEM (2011)

ICP-MS and speciation matured… (with introduction into legislation..??)

- Inorganic Arsenic in rice by 2012

…tabled max. permissible level of inorganic arsenic in rice (EU)

Table 1. Summary of available methods of analysis As in rice from various countries

Country	Total As	Inorganic As
Australia	ICP-MS – internationally validated	ICP-MS – not internationally validated
Brazil	ICP-MS and HG-AAS plus graphite furnace atomic absorption	None
China	ICP-MS and HG-AFS — national validation	HPLC method coupled with ICP-MS or HG-AFS – national validation
Colombia	ICP-MS and HG-AAS	None
European Union	Various – internationally validated	Various – internationally validated
Korea	No information	HPLC method coupled with ICP-MS
Japan	AOAC 986.15 (AAS)	HPLC coupled with ICP-MS – no information on validation status
US	ICP-MS – not internationally validated	HPLC coupled with ICP-MS – not internationally validated

→ Not enough data for legislation.

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Tabloid study with trigger value

Risk group: toddler and babies

- **Babies have 3 times intake per body weight as adults mg/kg/d**

and

- **Many baby products are based on rice**

→ **6 of 17 products could not be exported to China, who introduced a maximum permissible concentration of 150 µg/kg inorganic As**

AA Meharg, GX Sun, PN Williams, E Adomako, C Deacon, YG Zhu, J Feldmann, A Raab
Environ. Poll. (2008), **152**, 746-749

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Analytical challenges with new regulatory guideline

…although already simple methodology, methods need to be **faster** and **cheaper** and provide **species specific** information

Any intelligent fool can make things bigger,
more complex, and more violent.
It takes a touch of genius
-and a lot of courage - to move in the opposite direction.
-
(Albert Einstein)

… if you are not a genius just look in old papers….

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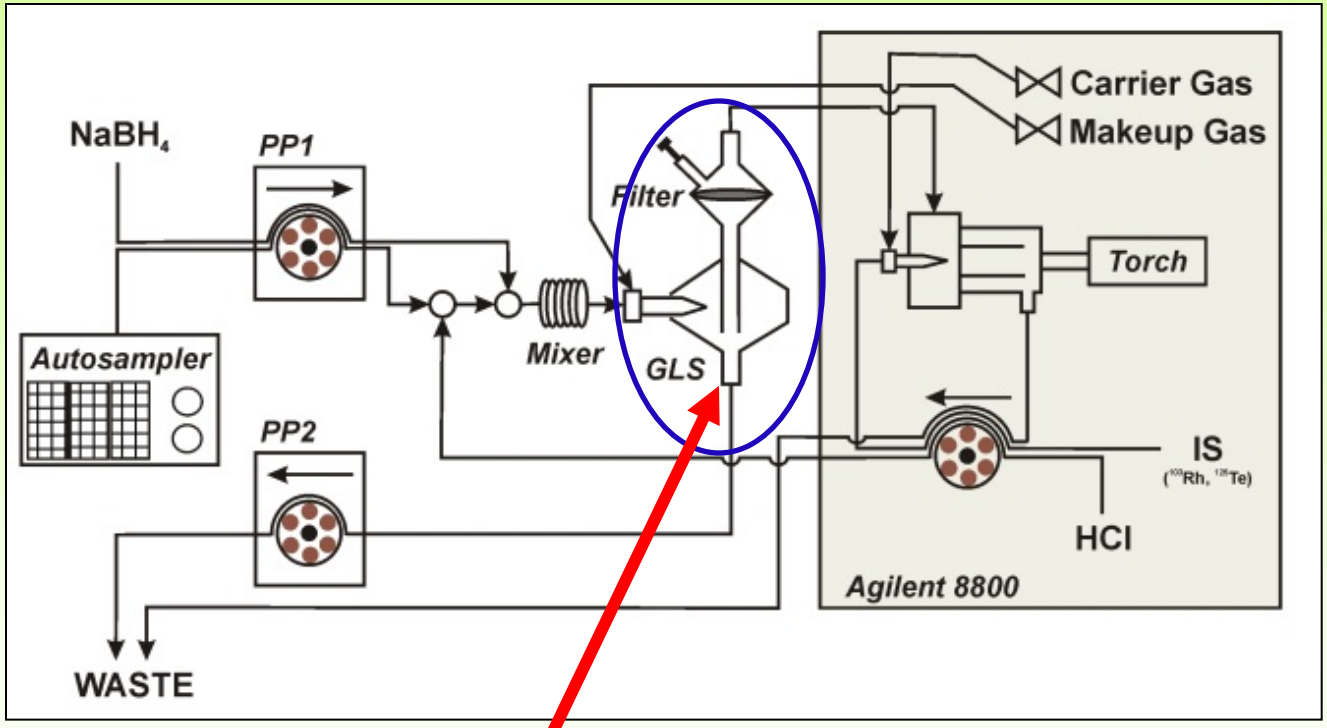
Analysis:
Hydride generation for iAs ONLY!

ARSENIC species + $NaBH_4$ + H_3O^+ →????

- iAs → AsH_3 bp: -55°C
- oAs (AsB, As-sugars etc) → Me_2As-R — Not volatile
- oAs (DMA) → Me_2AsH bp: 35°C — Not generated at low pH
- oAs (MMA) → $MeAsH_2$ bp: 2°C — Does not occur in rice or seafood

S. Musil, AH Petursdottir, H Gunnlaugsdottir, EM Krupp, J Feldmann
Analytical Chemistry (2014) 86**, 993-999**

Hydride Generation - ICPMS



Gas-Liquid-Separator:
Species specific volatilisation for inorganic As

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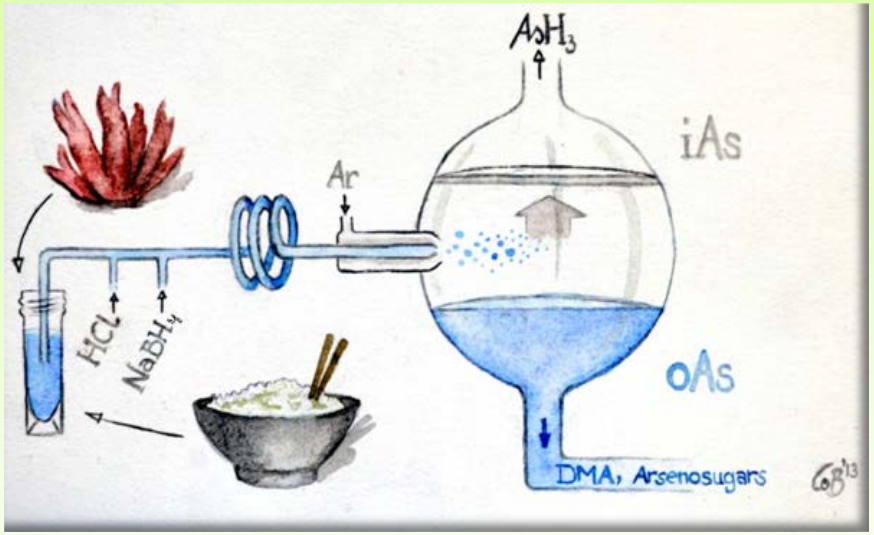
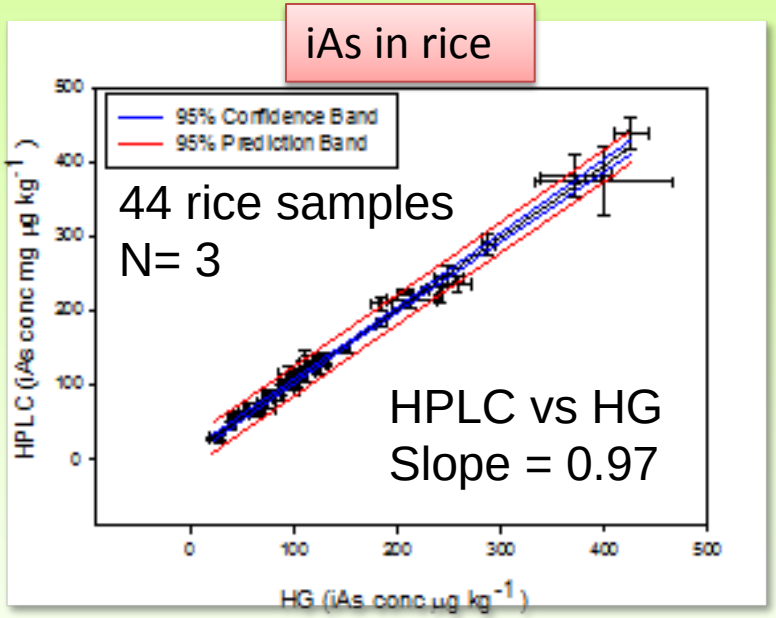
Speciation analysis *without* chromatography using discriminating HG-ICPMS





→ No need for HPLC, HG is selective enough,
→ faster and cheaper than using chromatography

S. Musil, AH Petursdottir, et al. Analytical Chemistry (2014) **86,** 993-999

AH. Petursdottir et al. Analytical Methods (2014) **6**, 5392-5396.

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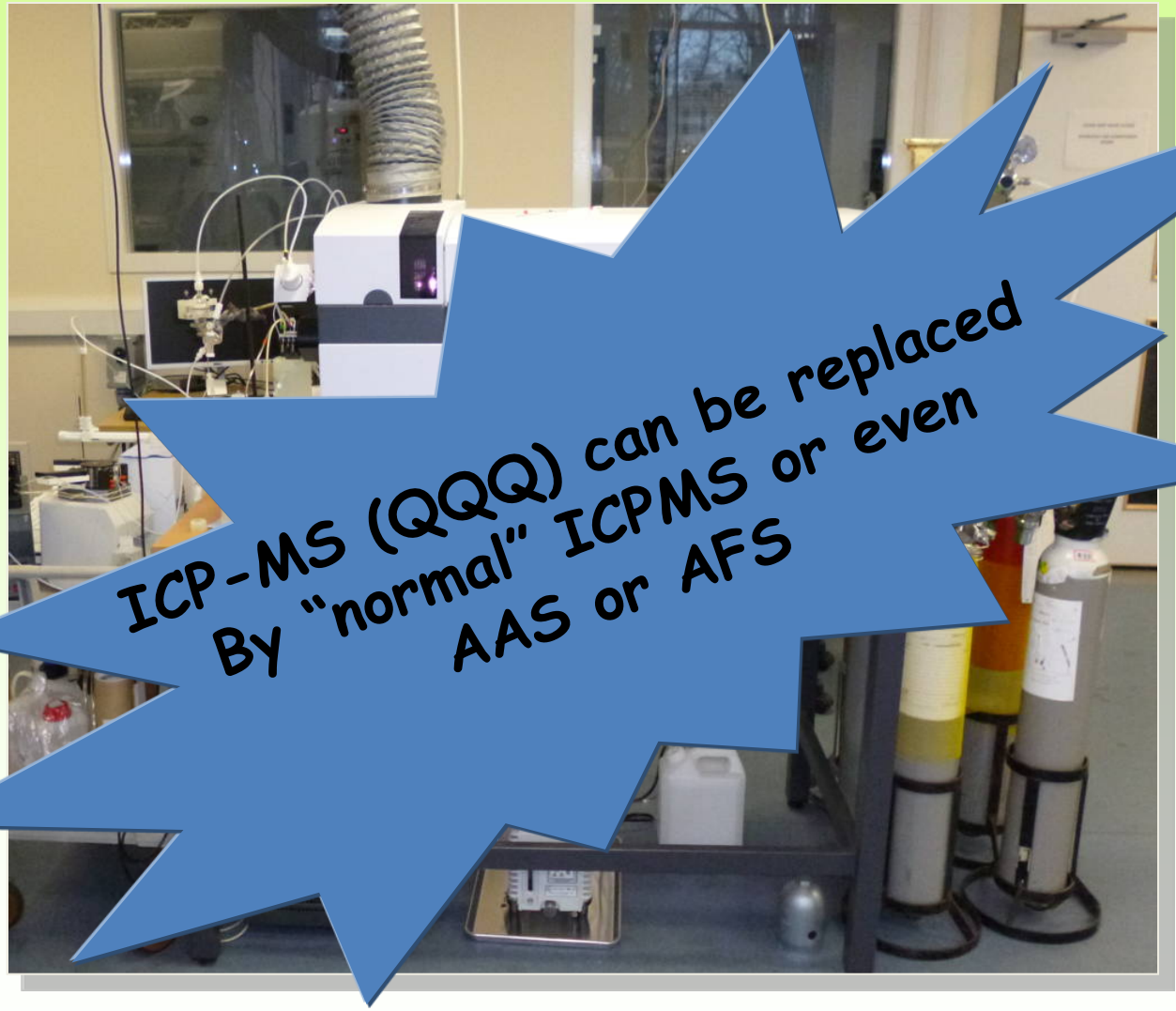
Agilent 8800 QQQ (ICPMS)



→ Collaboration with Agilent (Food Group)

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New legislation for inorganic Arsenic in rice

"COMMISSION REGULATION (EU) 2015/1006 of **25 June 2015** amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs"

Arsenic (inorganic)	As (mg/kg)
Non-parboiled milled rice (polished or white rice)	0.20
Parboiled rice and husked rice	0.25
Rice waffles, rice wafers, rice crackers and rice cakes	0.30
Rice destined for the production of food for infants and young children	**0.10**

http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv%3AOJ.L_.2015.161.01.0014.01.ENG

An important step forward..

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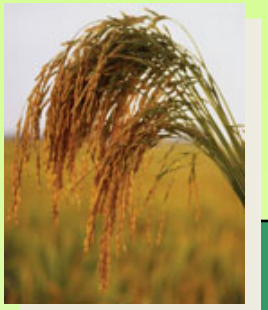
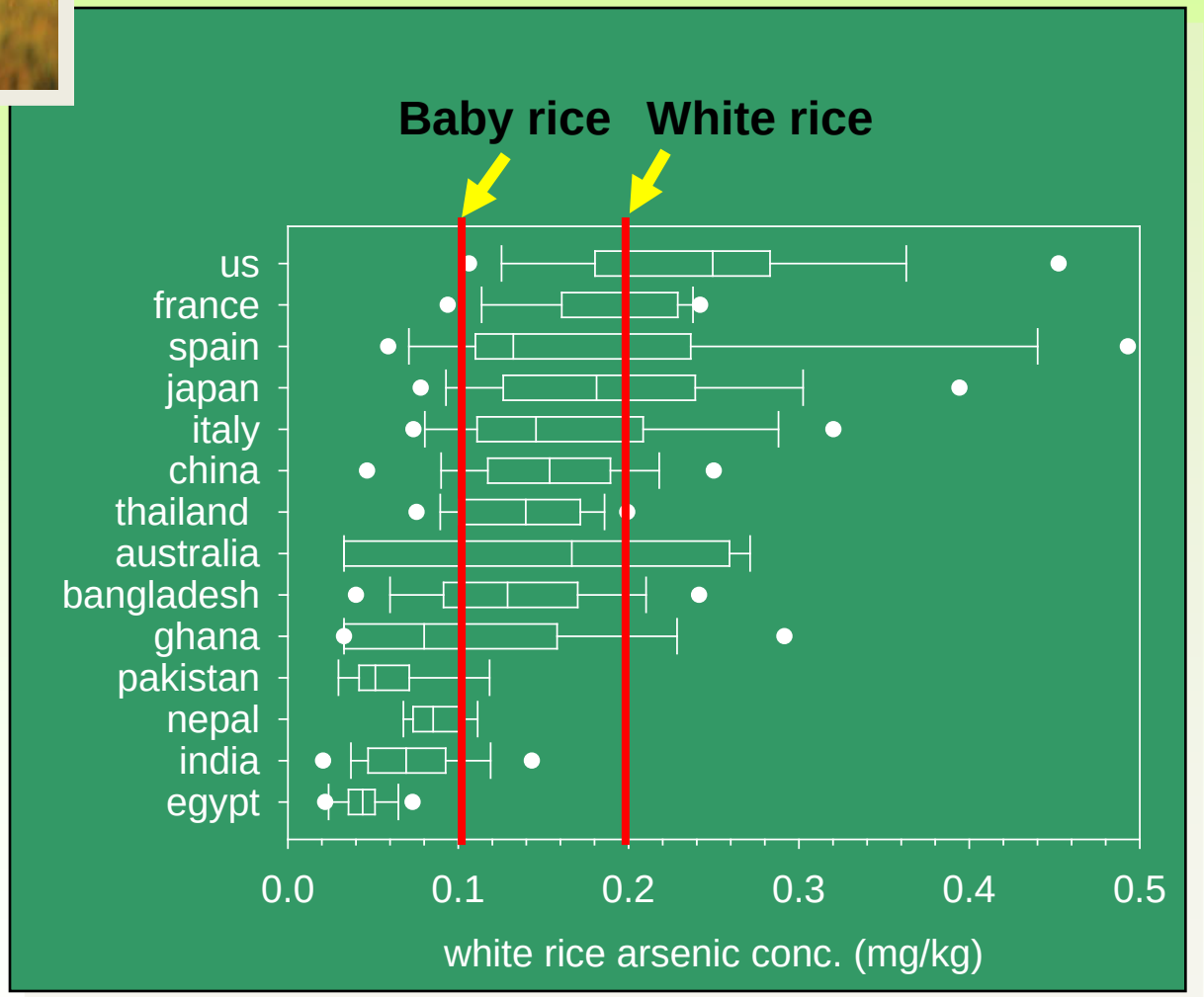


Arsenic in rice surveys

iAs of ~1000 rice samples from different origin



Meharg et al. ES&T (2009) **43**, 1612

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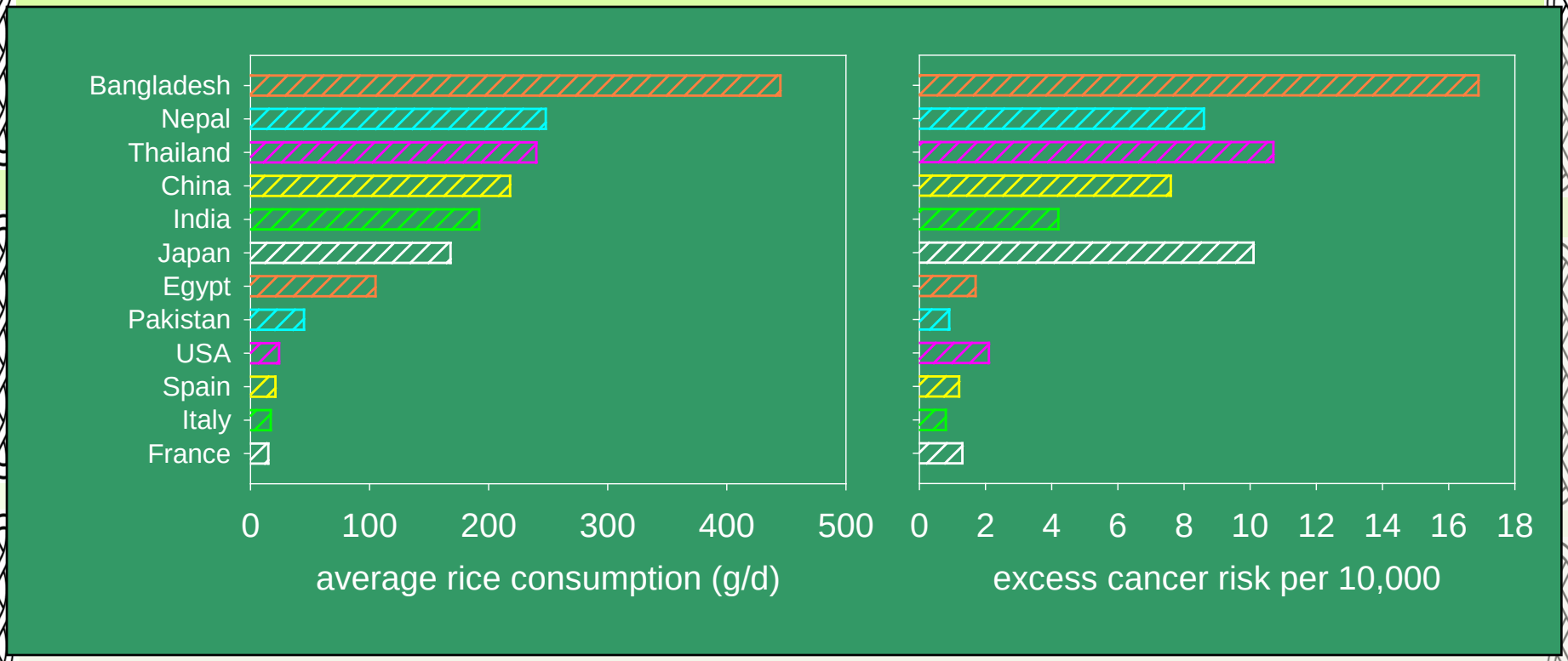
Excess cancer risk from inorganic arsenic in rice:

based on country specific consumption rates;



WHO: 10 µg As / day; → for Bangladesh: ~100 µg As;
..whilst observing the new EU legislation @ 0.20 mg/kg in rice…

Meharg et al. Environ. Sci. Technol. (2009) **43**, 1612

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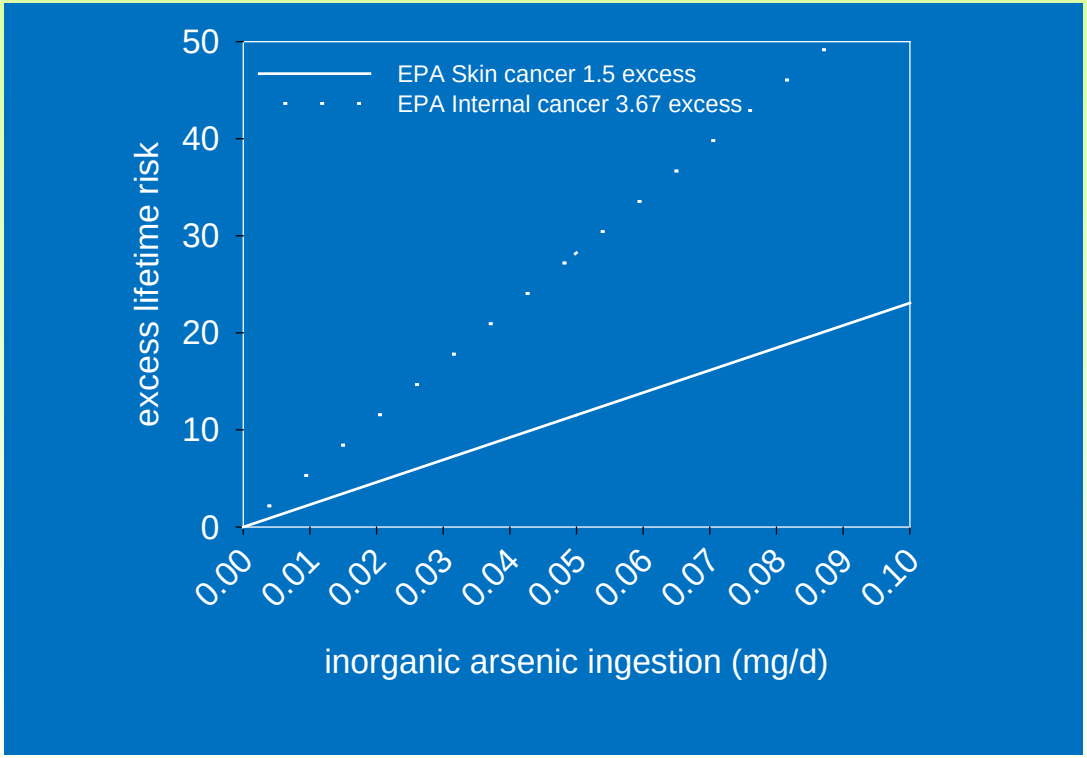
TOXICOLOGICAL IMPLICATIONS:
EPA cancer slopes excess lifetime risk per 10,000:



→US EPA target for any given source should not exceed a cancer risk of 1 in 10,000 !

Excess lifetime risk per 10,000 based on consumption of 1 L water @ 10 µg/L per day, or a total of 10 µg As / day.

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Can methods be even simpler, and applied on-site, in rural areas?

Aim:

1) develop a field based method for detection of ***inorganic As*** in rice grains
2) obtain results ***within one hour*** using a field test kit without electricity

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The principle

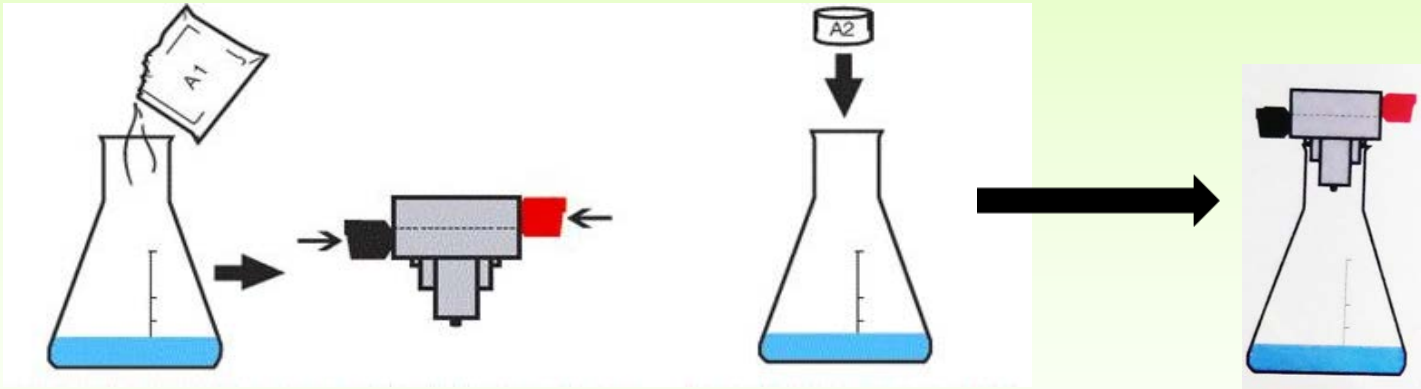
- based on the Gutzeit Method (19th century technology):

$$2H_3AsO_4 + 2H_3O+ + 2NaBH_4 \rightarrow 2AsH_3 + 2B(OH)_3 + 2H_2O + 2Na^+$$

$$AsH_3 + HgBr_2 \rightarrow H_2As–HgBr + HBr$$

Limit of detection: 3-4 µg/L in 50 mL solution

Reagents: sulphamic acid (A1) and sodium borohydride (A2)



OR

E. Bralatei et al. Analytical Chemistry (2015) 87, 11271

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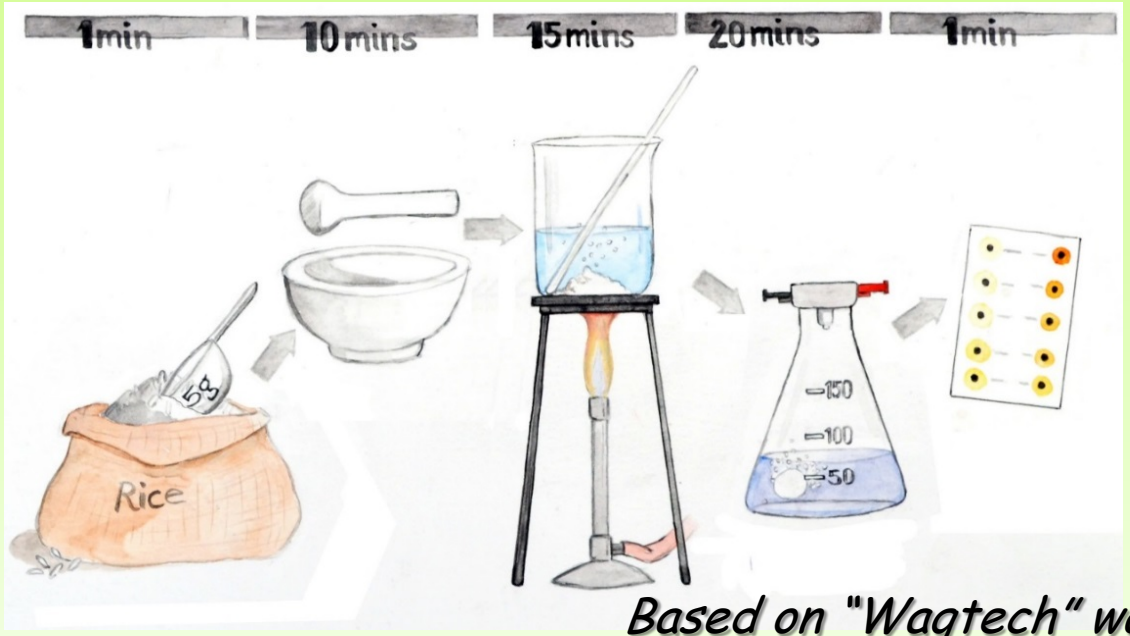
Main idea and experiments…..



Based on "Wagtech" water analysis kit

Field method optimisation (Extraction)

- Steeping time, boiling time
- (extraction was 1 % HNO_3 as for HPLC-ICPMS analysis)
- Sampling, homogenisation, etc.

Method Validation

- Method validation using HPLC-ICP-MS as reference method

E. Bralatei et al. Analytical Chemistry (2015) 87, 11271

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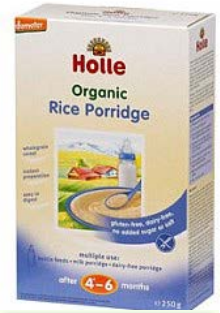
Samples

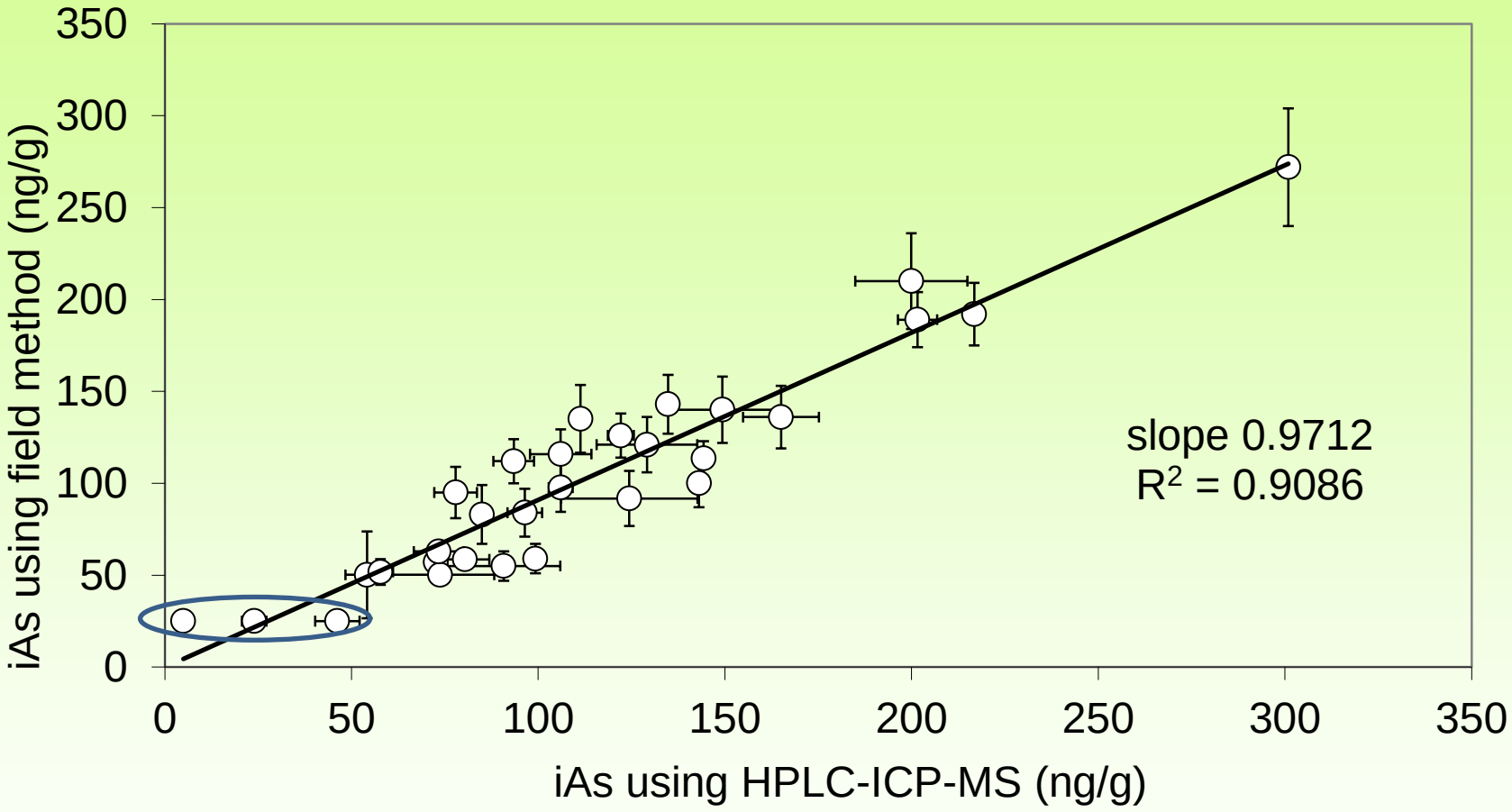
30 Rice samples and rice based products were obtained from various retailers in Aberdeen

- Rice grain samples were of different cultivars and different country of origin
- Rice based products including rice noodles, rice paper and rice flour
- Rice based baby food from different brands



E. Bralatei et al. Analytical Chemistry (2015) 87, 11271

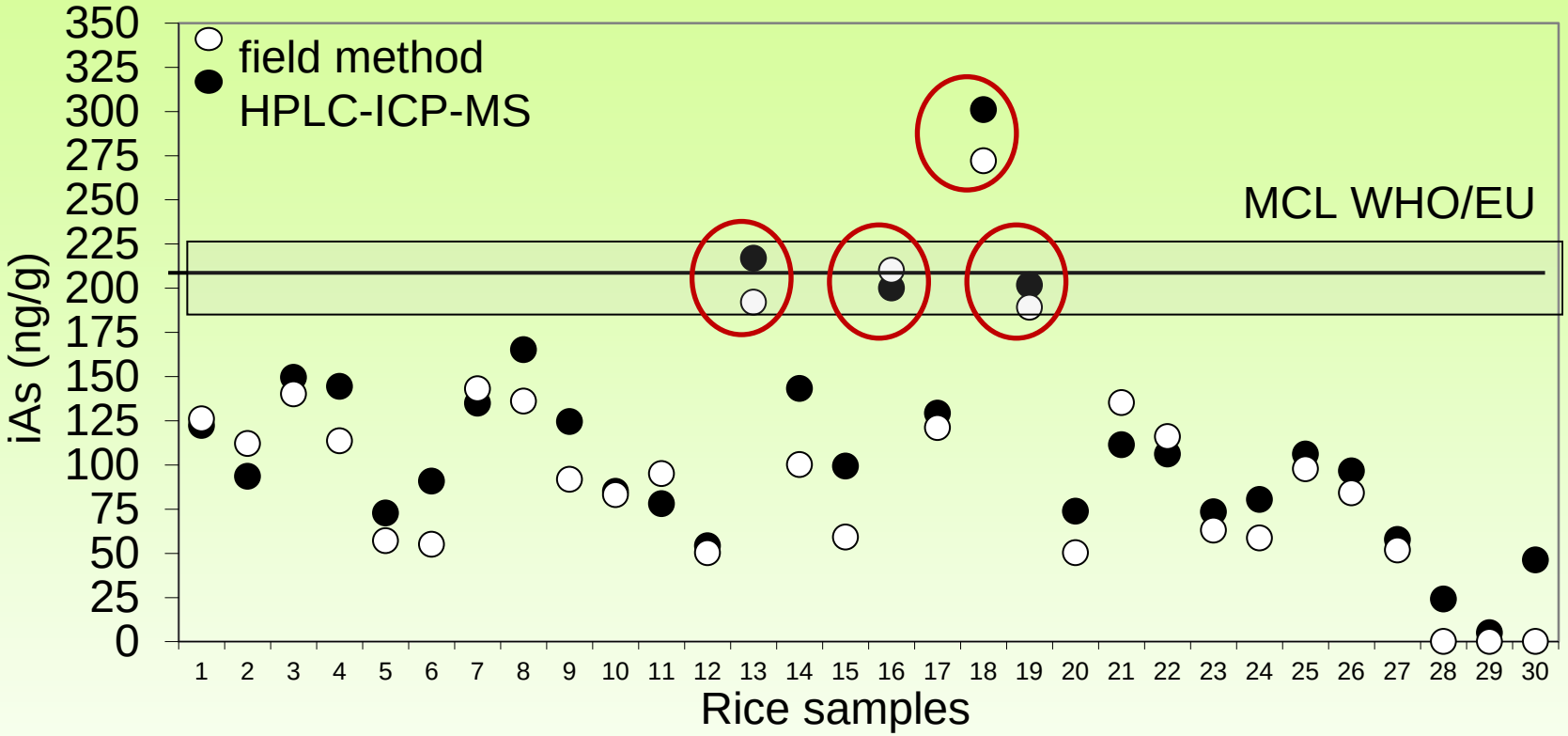
Field method validation



- A slope of 0.97 with a R^2 of 0.9086 indicates a good correlation between the field method and reference method HPLC-ICP-MS.

E. Bralatei et al. Analytical Chemistry (2015) 87, 11271

Sample screening



Based on the WHO maximum contaminant limit of 200 µg/kg with ±12% RSD from the field method,

- 13% (4 out of 30) of samples are above the recommended limit
- No false positive or negative results are recorded

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Why does arsenic accumulate in rice ?

- Herbicides in US (PN Williams et al. ES&T (2007), **41**, 2178)
- Mining in China (YG Zhu et al. ES&T (2008), **42**, 5008)
- Irrigation in Bangladesh (PN Williams et al. ES&T (2006), 40, 4903)
- Cultivars in Bangladesh (GJ Norton et al. ES&T (2009), **43**, 6070)
- Cultivars (phenotype) and site interactions in India, Bangladesh, China (GJ Norton et al. ES&T (2009), **43**, 8381)

→ We wanted to understand translocation of As in rice

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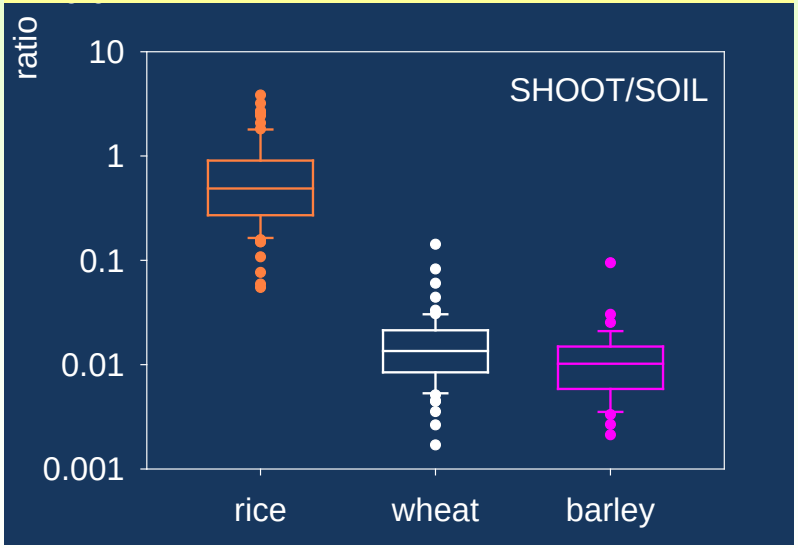
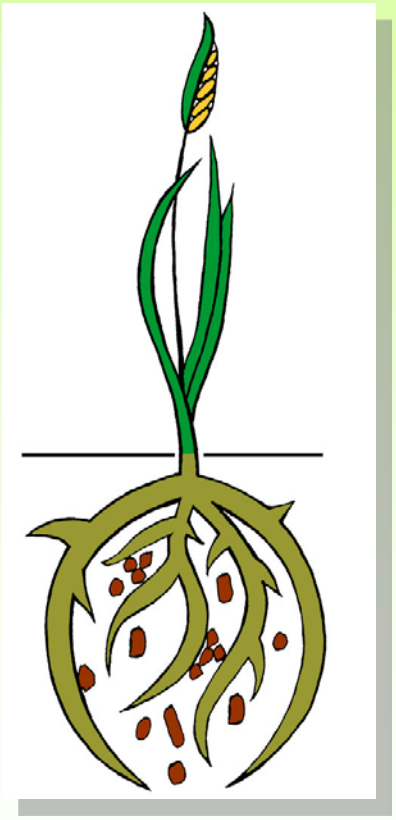
Translocation of As in plants

Rice takes up more Arsenic from the soil than other cereals!!





PN Williams et al. ES&T (2007) 41, 6854

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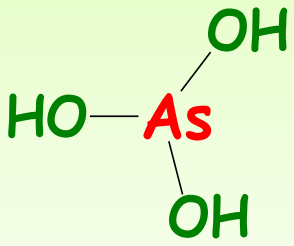
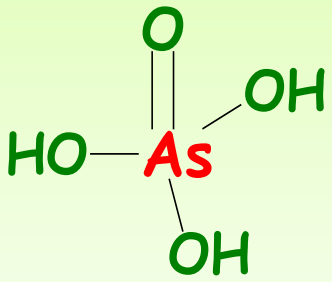
As-speciation in soil porewater is important

These inorganic As species are abundant in pore water:

As(III)



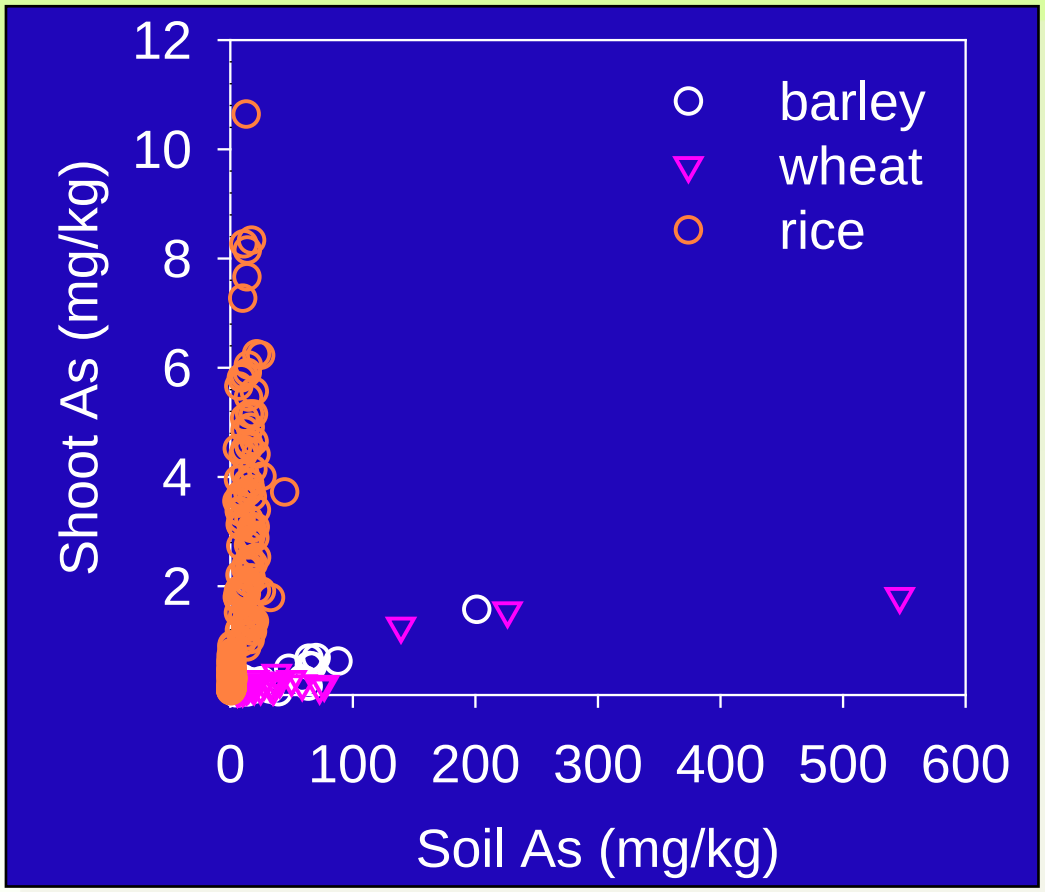
anaerobic conditions



As(V)

aerobic conditions (oxidised)

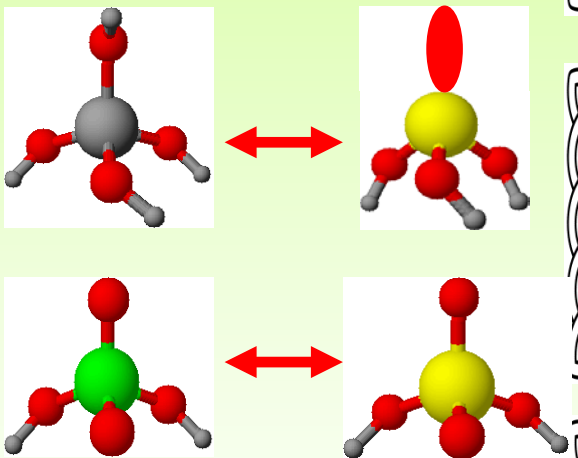
As accumulation in rice: high but variable



Ma et al. PNAS (2008), 105, 9931

Nutrient uptake mistaken?

$Si(OH)_4$ $As(OH)_3$



$H_2PO_4^-$ $H_2AsO_4^-$

→ Less As uptake in aerobic conditions

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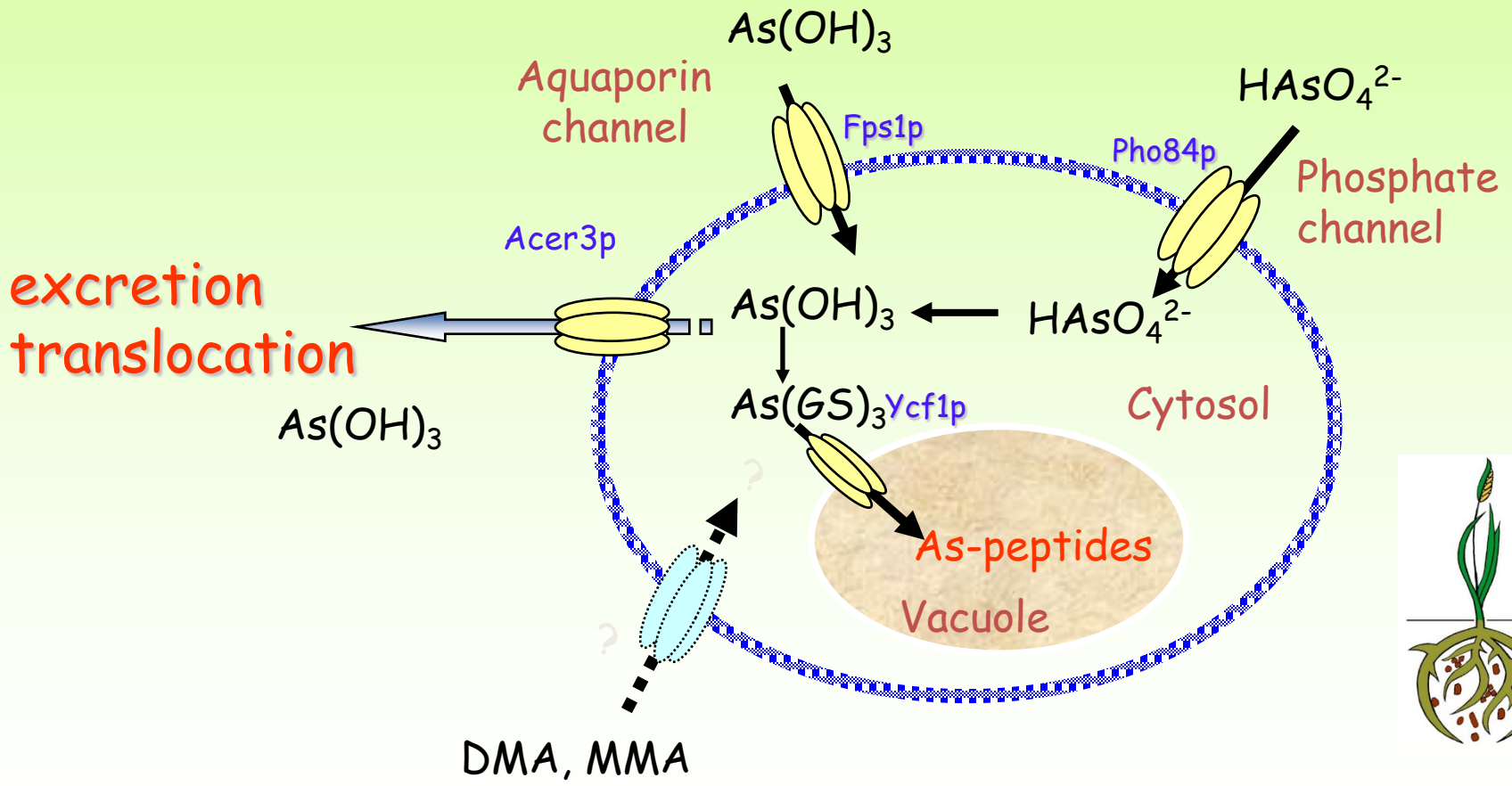
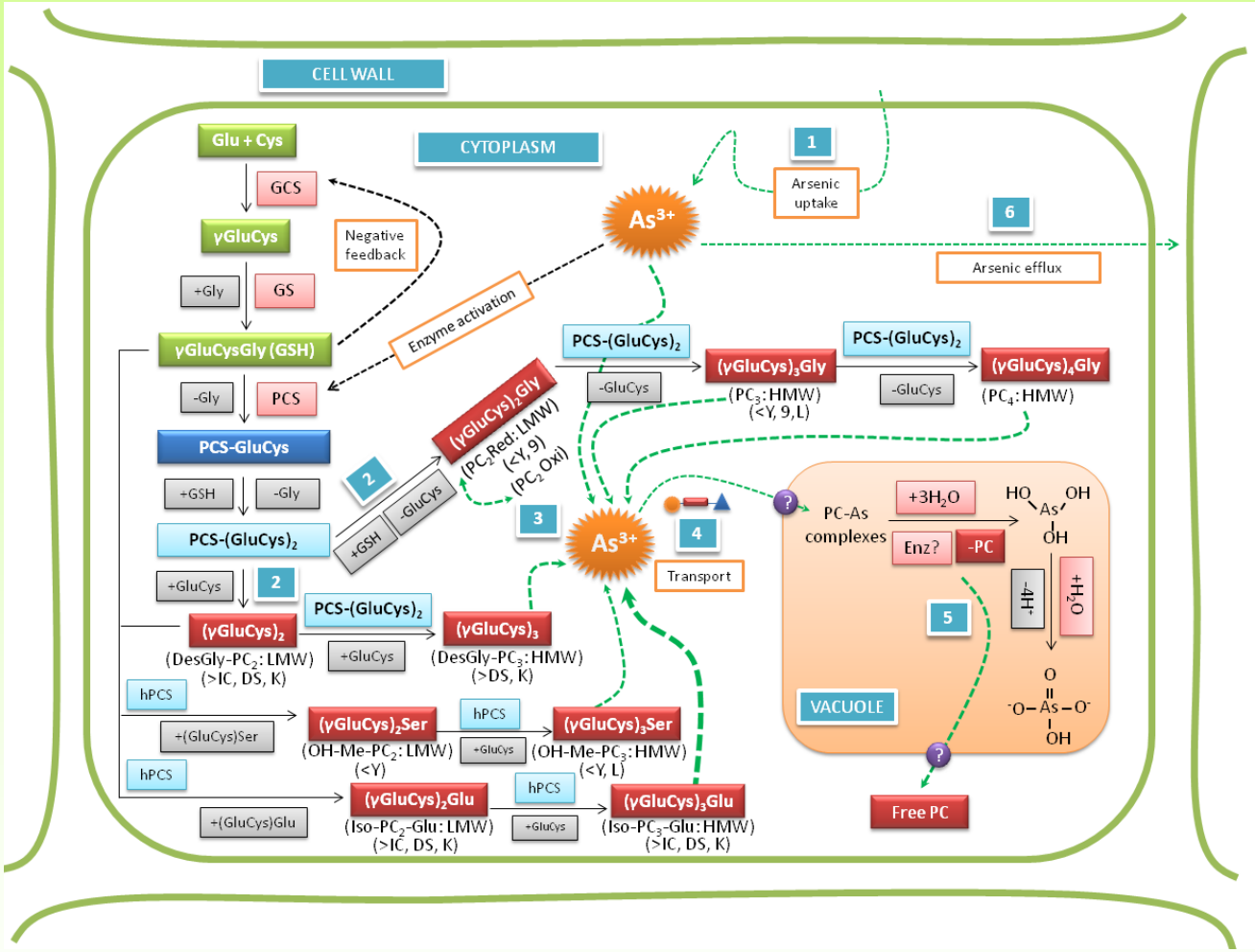
As uptake, accumulation and excretion mechanism



adopted model from yeast (B. Rosen)

Arsenic and Phytochelatins in Rice



B.L. Batista, M Nigar, A Mestrot, BA Rocha, F Barbosa Jr, A.H. Price, A Raab, J Feldmann

Journal of Experimental Botany *(2014) doi:10.1093/jxb/eru018*

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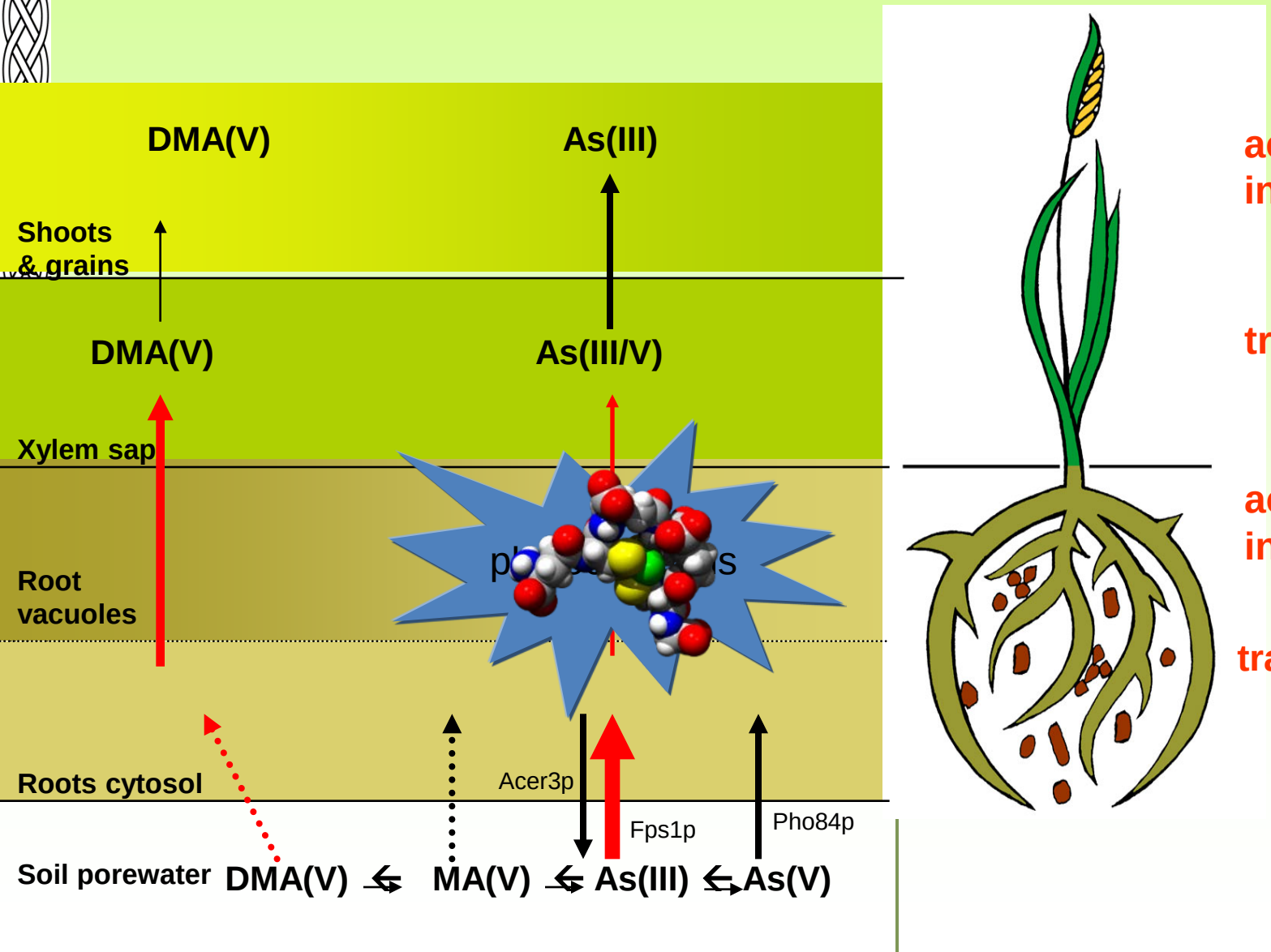
WJ Liu, BA Wood, A Raab, SP McGrath, FJ Zhao, J Feldmann *Plant Physiology* (2010), 152, 2211-2221

Uptake and translocation of As in rice



accumulation in shoots

translocation

accumulation in roots

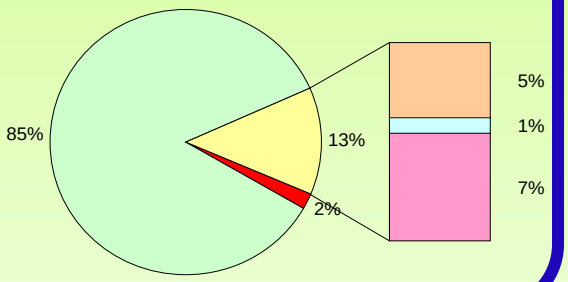
transformation

uptake

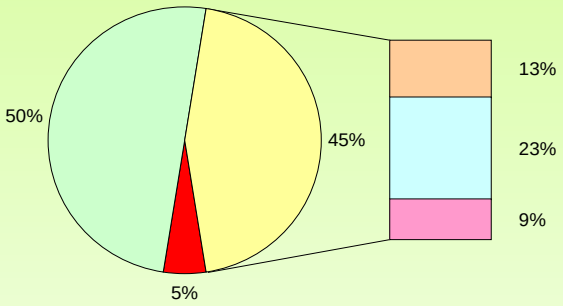
Emerging analytes: focus on Arsenolipids

Fish

1 mg/kg



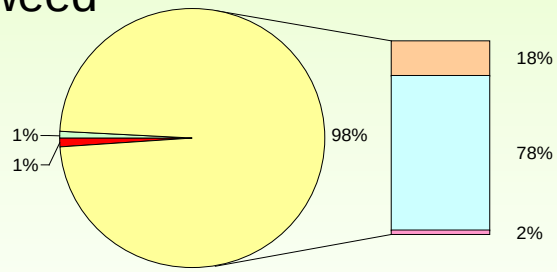
Molluscs



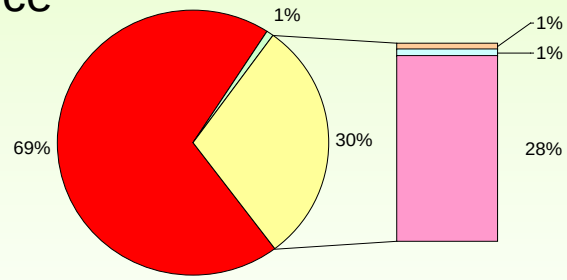
3 mg/kg

Seaweed

100 mg/kg



Rice



0.3 mg/kg

toxic (iAs)

non-toxic (AsB)

potentially toxic

arsenic fractions

fat-soluble As (e.g. As-Lipids)

arsenosugars

other organoarsenicals

potentially toxic

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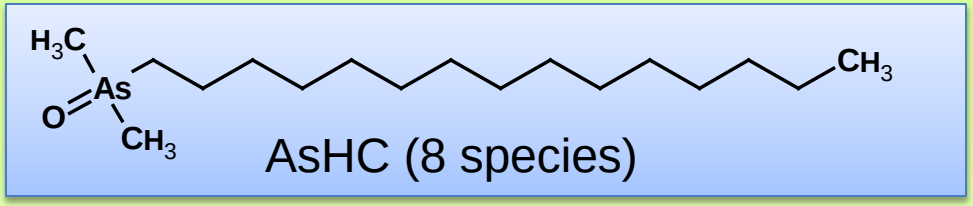
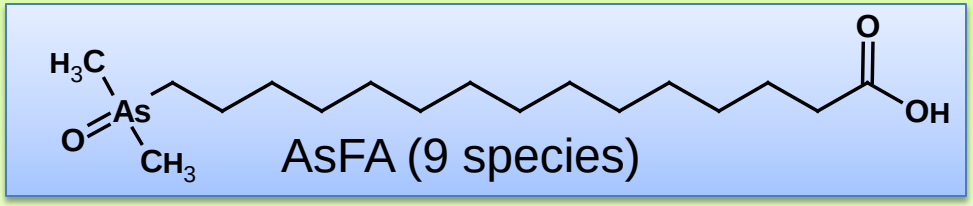
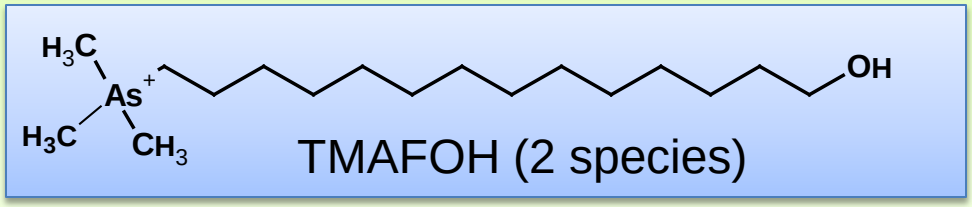
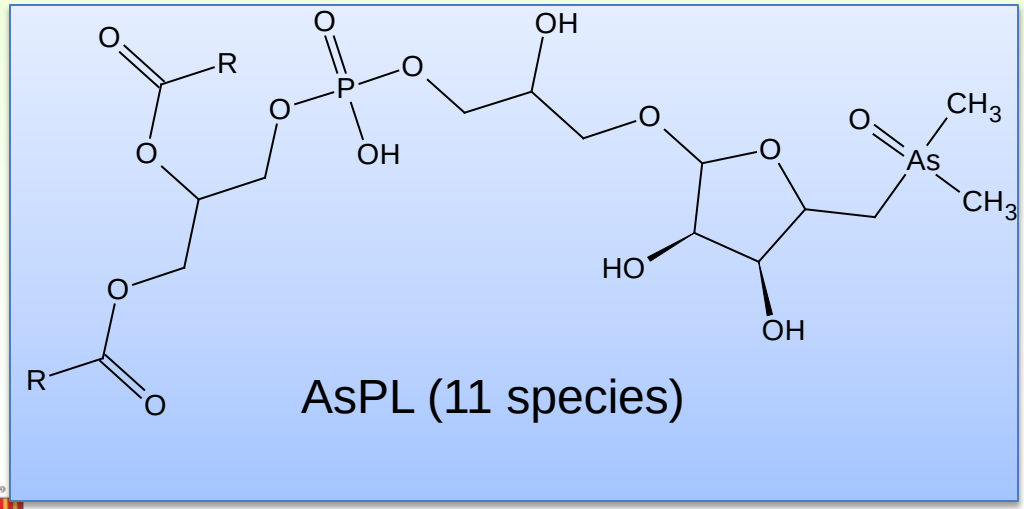
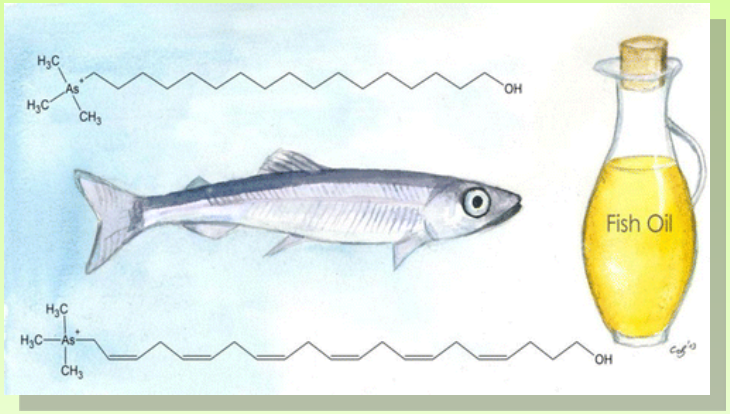
Classes of identified arsenolipids (AsLp)











+ unstable AsLp

A. Raab, et al
Anal. Chem (2013) **85**, 2817.

KO Amayo, et al.
Anal Chem (2013) **85**, 9321-9327

KO Amayo et al.
Talanta (2014) **118**, 217-223.

KO Amayo et al.
JTEMB (2014) …..

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Pilot whales as top predators

mass stranding, NE Scotland Sept 2nd 2012

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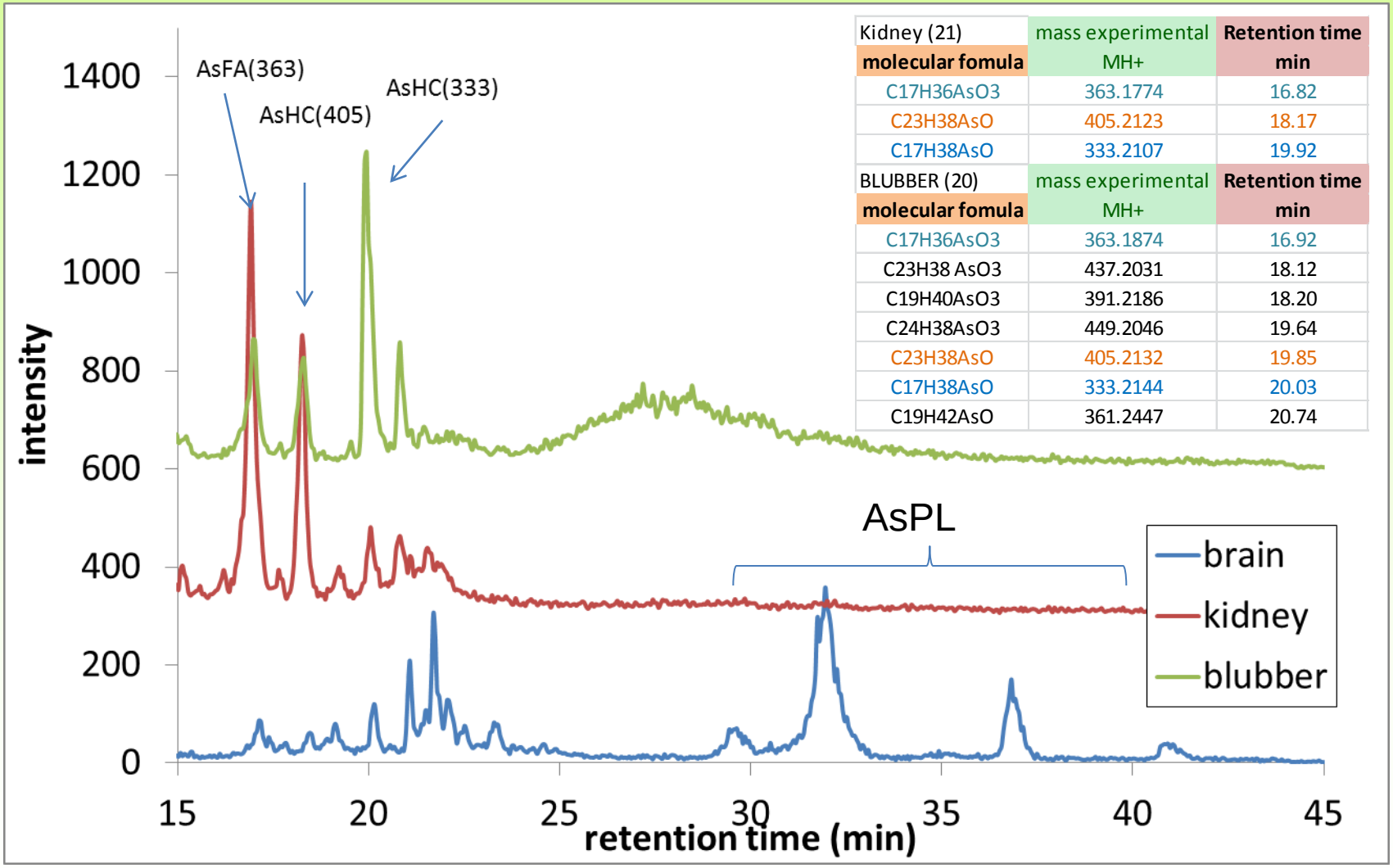
AsFA, AsHC and AsPL in pilot whale tissues

RP-HPLC-ICPMS/ESI-MS chromatograms

Kidney (21)	mass experimental	Retention time
molecular fomula	MH+	min
C17H36AsO3	363.1774	16.82
C23H38AsO	405.2123	18.17
C17H38AsO	333.2107	19.92
BLUBBER (20)	mass experimental	Retention time
molecular fomula	MH+	min
C17H36AsO3	363.1874	16.92
C23H38 AsO3	437.2031	18.12
C19H40AsO3	391.2186	18.20
C24H38AsO3	449.2046	19.64
C23H38AsO	405.2132	19.85
C17H38AsO	333.2144	20.03
C19H42AsO	361.2447	20.74



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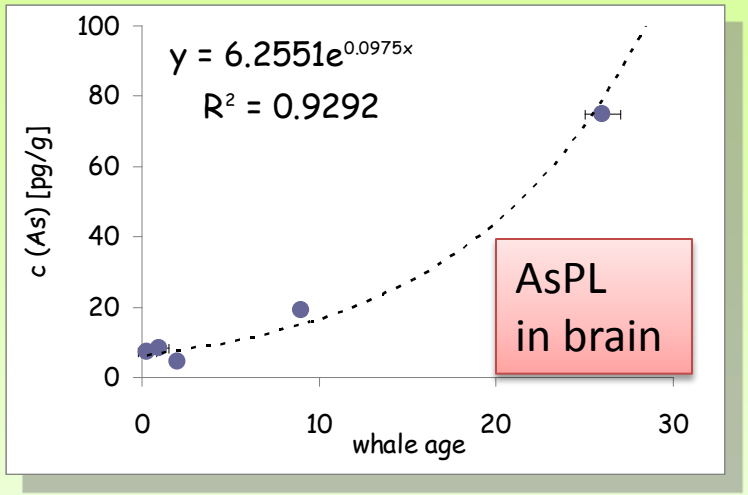
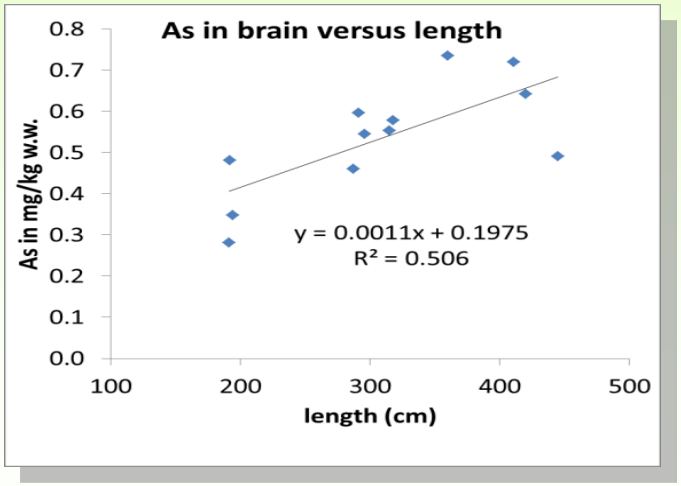
AsLp in kidney, liver and brain

- Presence of AsLp in **all** tissues
  - AsHC and AsFA in kidney and liver
  - AsHC, AsFA and AsPL in brain
  - Even total As in brain shows a bioaccumulation effect





Evidence for toxicity:

S. Meyer et al. (2014), "Arsenic-containing hydrocarbons are toxic in the *in vivo* model *Drosophila melanogaster*" *Metallomics*, 2014, Nov;6(11):2010-4,

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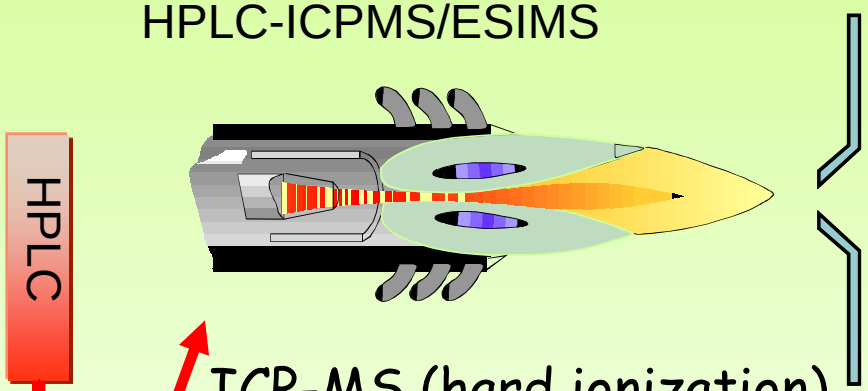
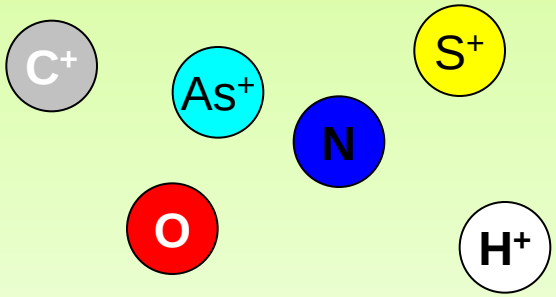
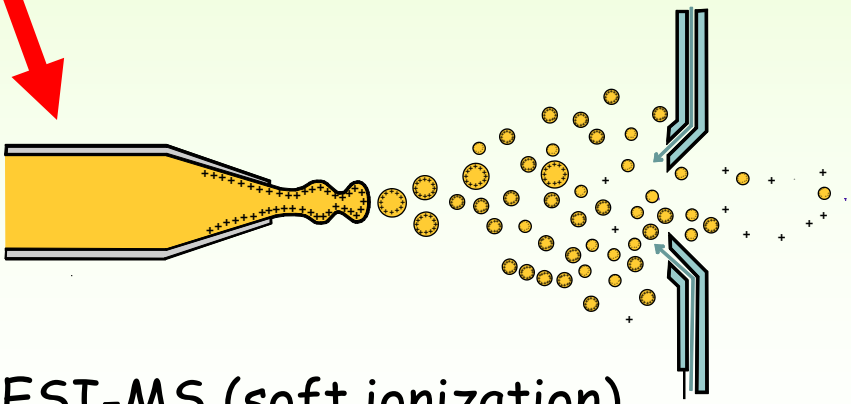
However, for these types of analysis we need more sophisticated methods…

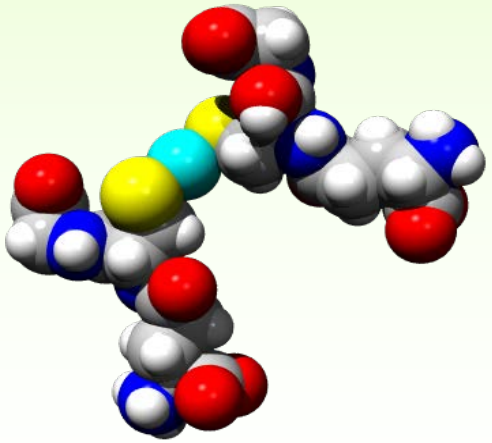
HPLC-ICPMS/ESIMS



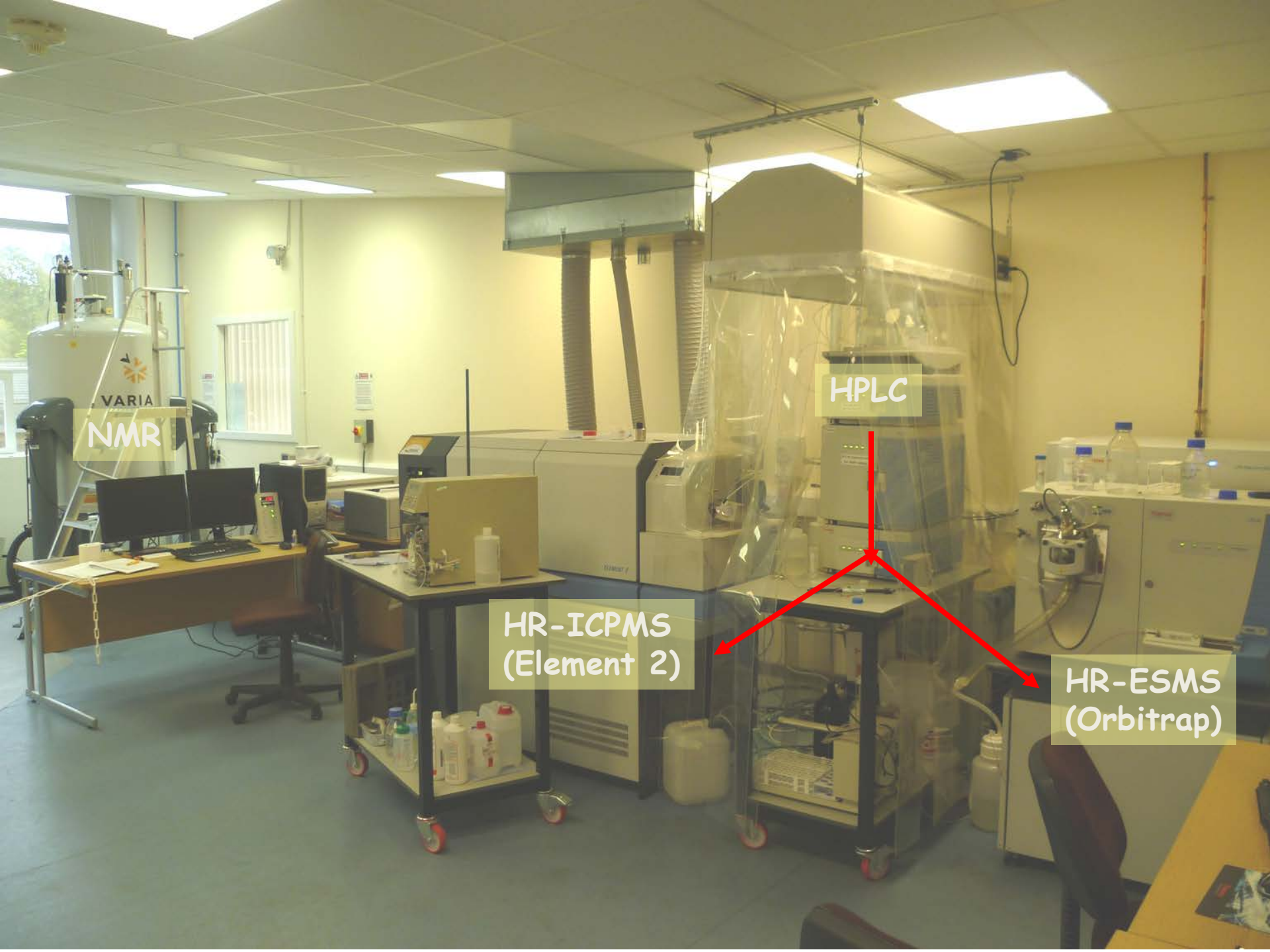


ICP-MS (hard ionization)
Element specific detection





ESI-MS (soft ionization)
molecule specific detection


NMR
HPLC
HR-ICPMS
(Element 2)
HR-ESMS
(Orbitrap)

Take home message

- HG-ICP-MS for inorganic As speciation paved the way to n EU legislation on iAs in rice
- Arsenic PC-complexes can explain iAs transport and accumulation in rice grain
- A huge variety of arsenolipids can be found in fish
- Sophisticated HPLC-ICP-MS/ESI-MS is needed here
- Cytotoxic arseno-hydrocarbons were found in the brain of pilot whales

Thanks for listening!

Scottish Funding Council
Promoting further and higher education

Asta Petursdottir

Andrea Raab

Juliana MO Souza

Eva M Krupp

Kenneth O Amayo

Chris Newcombe