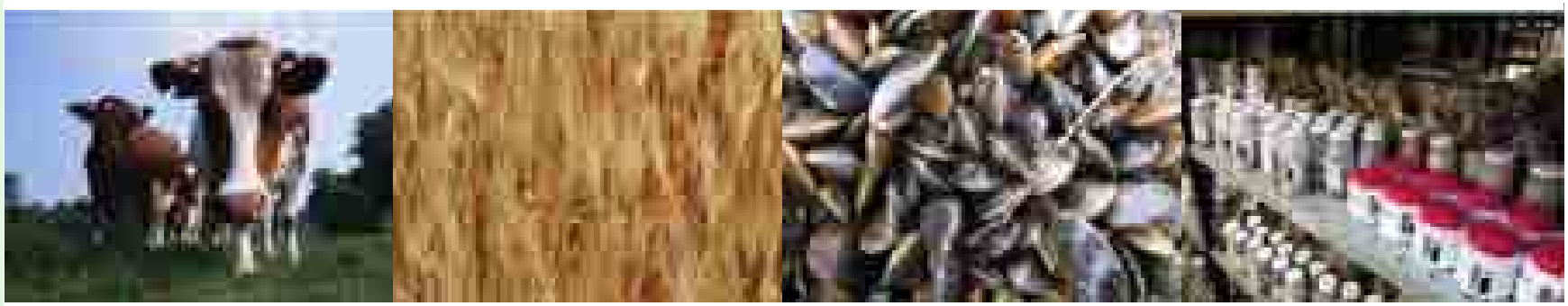
Rapid tests for chemical contaminants in seafood and fish feed – the CONffIDENCE research project (EU-FP7)

Jacob de Jong, RIKILT – Institute of Food Safety, Wageningen UR (NL)

www.conffidence.eu



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Introduction (1)

- Public concerns about presence of chemical contaminants in food and feed

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Introduction (2)

SCIENTIFIC AMERICAN™

Winner of the 2011 National Magazine Award for General Excellence

Gulf Seafood Officially Safe, But Questions and Oil Linger

Testing of shrimp, crabs and fish, among other seafood harvested in the Gulf of Mexico, continues

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Introduction (3)

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Introduction (4)

- Globalisation of trade in food and feed ingredients

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Introduction (5)

What is the challenge ?

- Fast and cost-effective screening tests for contaminants in food and feed:
  - Product acceptance by companies
  - Official control

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CONffIDENCE in a nutshell

CONtaminants in *f*ood and *f*eed:

Inexpensive DEtectioN

for Control of Exposure

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CON*ff*IDENCE passport

- FP7 Collaborative Project first call “Food, Agriculture & Fisheries, and Biotechnology”
- Duration: May 2008 – April 2012
- 16 partners from 10 countries, representing universities, research institutes, industry and SMEs
- Budget: 7.5 Mio €
- Coordinator: RIKILT - Institute of Food Safety, part of Wageningen UR (NL)

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The commodities

Food	&	Feed
Fish/shellfish		**Fish feed**
Cereals		Cereal-based feed
Potatoes/vegetables		
Honey		
Eggs		
Meat		
Dairy products		

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The target contaminants

- POPs (Persistent Organic Pollutants):
  - dioxin-like PCBs
  - brominated flame retardants
  - polycyclic aromatic hydrocarbons (PAH)
- Perfluorinated compounds (PFCs)
- Pesticides
- Veterinary drugs: - antibiotics
  - coccidiostats
- Heavy metals speciation: inorganic arsenic, methyl mercury
- Biotoxins: - alkaloids
  - marine biotoxins
  - mycotoxins

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Target contaminants for fish (feed)

- POPs: - dioxin-like PCBs
  - brominated flame retardants
  - polycyclic aromatic hydrocarbons (PAH)
- Perfluorinated compounds (PFCs)

- Veterinary drugs: - antibiotics
- Heavy metals speciation: inorganic arsenic, methyl mercury
- Biotoxins:
  - marine biotoxins
  - mycotoxins (cereals)

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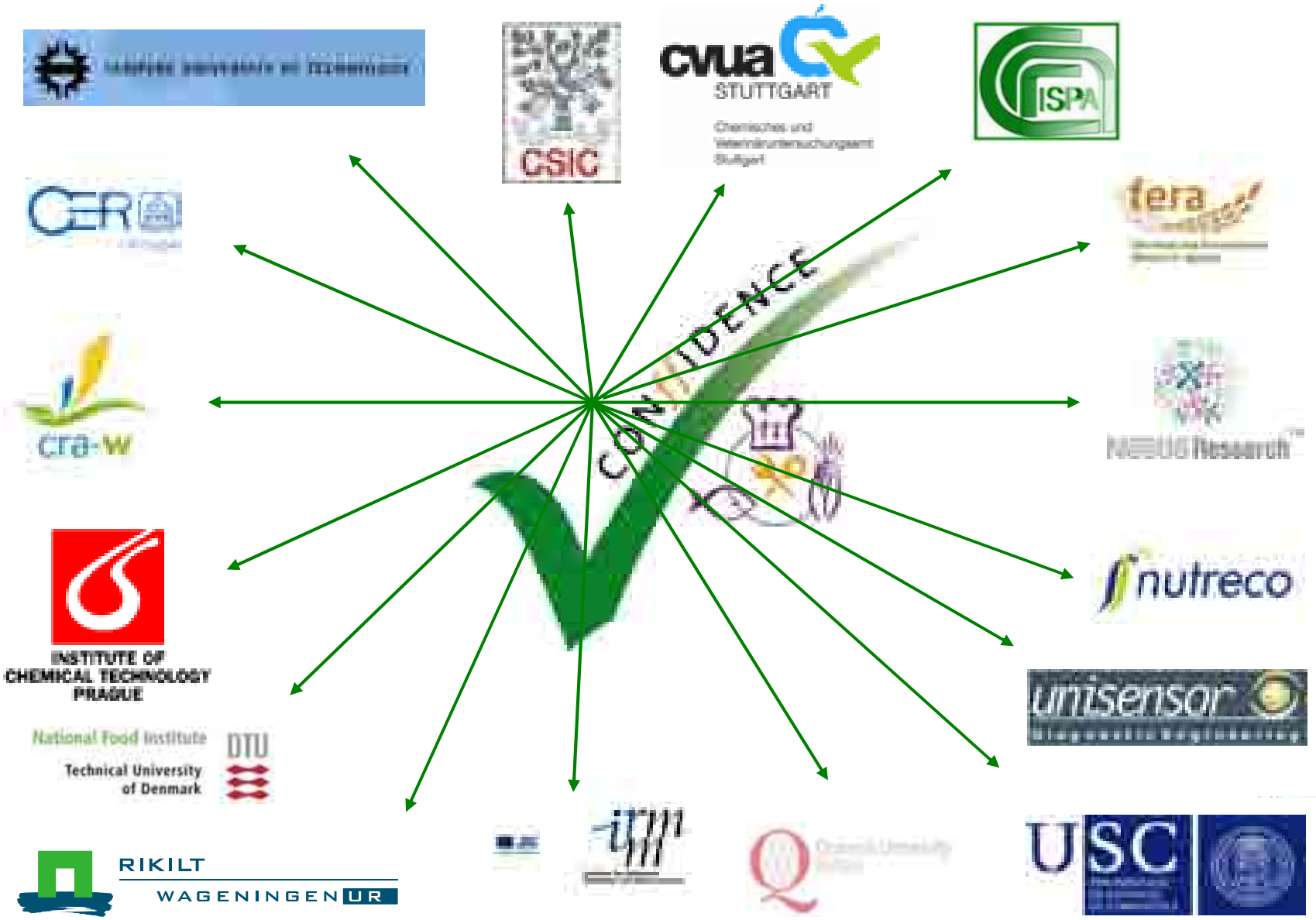
The consortium



Current state of progress

- Production and characterization of test materials
- Production of high-quality binders (mainly antibodies)

↓

- Development of the simplified multiplex assays (buffer)
- Development of simplified sample preparation

↓

- Prototype assay protocol
- In-house validation, comparison vs instrumental methods

↓

- Small-scale interlab studies

Year 1

Year 4

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Results for (shell)fish and fish feed

Example 1:

POPs: - dioxin-like PCBs + metabolites
- brominated flame retardants
- polycyclic aromatic hydrocarbons (PAHs)

- Simplified and rapid determination of PCBs, PBDEs and PAHs in fish, fish feed and seafood integrated into a single method

Jana Hajslova and Jana Pulkrabova

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Integrated sample preparation

BFR | PCB | PAH | Non-*ortho* PCB

Extraction
Shaking (H_2O + ethylacetate)

Isolation
10 min

Partition (transfer into organic phase)
induced by $MgSO_4$ + NaCl

Clean up
Silica minicolumn

Clean up
30 min

Identification & quantification
GC-TOFMS (EI)
GC GC-TOFMS (EI)

Identification & quantification
1 h

6 SAMPLES / < 1 HOUR

SINGLE GC INJECTION

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INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE

Oil Spill - Gulf Of Mexico 2010

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Call for Methods

Methods for Measurement of Polycyclic Aromatic Hydrocarbon (PAH) Compounds in Gulf of Mexico Seafood

AOAC INTERNATIONAL is inviting method developers to submit methods for consideration and possible evaluation through the AOAC *Official Methods*SM program. Prospective methods must be able to quantify polycyclic aromatic hydrocarbon (PAH) “seafood”. Acceptable methods must be able to demonstrate a **Limit of Quantification of 1 ppb** (ng/g) for benzo(a)pyrene in seafood. Currently accepted analytical methods require 96 to 120 hours to complete. Evaluation of analytical methods that **significantly reduce the time-to-signal** (including sample preparation and extraction**) is a primary goal of this call for methods**.

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Target analytes – AOAC study

16 EU PAHs		EPA PAHs + methylated homologues	
Benz[a]anthracene	BaA	2-Methylanthracene	2-MA
Benzo[b]fluoranthene	BbFA	1-Methylchrysene	1-MC
Benzo[k]fluoranthene	BkFA	3-Methylchrysene	3-MC
Benzo[j]fluoranthene	BjFA	1-Methylnaphthalene	1-MN
Benzo[c]fluorene	BcFL	2-Methylnaphthalene	2-MN
Benzo[ghi]perylene	BghiP	1-Methylphenanthrene	1-MPH
Benzo[a]pyrene	BaP	1-Methylpyrene	1-MP
Chrysene	CHR	1,7-Dimethylphenanthracene	1,7-DMP
Cyclopenta[cd]pyrene	CPP	2,6-Dimethylnaphthalene	2,6-DMN
Dibenz[a,h]anthracene	DBahA	Acenaphthene	AC
Dibenzo[a,e]pyrene	DBaeP	Acenaphthylene	ACL
Dibenzo[a,h]pyrene	DBahP	Anthracene	AN
Dibenzo[a,i]pyrene	DBaiP	Benzo[e]pyrene	BeP
Dibenzo[a,l]pyrene	DBalP	Dibenzothiophene	DBT
Indeno[1,2,3-cd]pyrene	IP	Fluoranthene	FA
5-Methylchrysene	5-MC	Fluorene	FL
		Naphthalene	NA
		Phenanthrene	PHE
		Pyrene	PY

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INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE

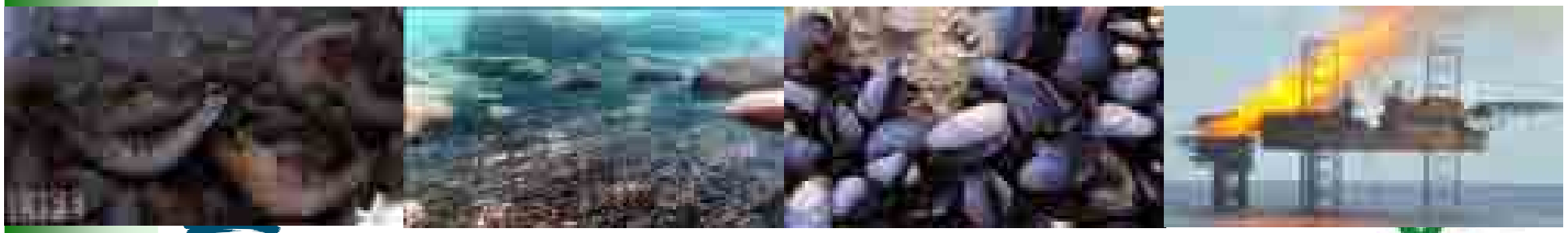
NEWS FLASH

PAH Update: Candidate Method to Enter Collaborative Study

Due to the urgent need for rugged, reliable methods to determine polycyclic aromatic hydrocarbon (PAH) compounds in seafood from the Gulf, AOAC expedited a process that, ultimately, led to a candidate method ready for AOAC validation. AOAC facilitated a stakeholder panel and working group meetings, established a fitness-for-purpose statement, issued calls for methods and collaborators, evaluated available methodology purported to meet fitness for purpose; and selected the best candidate method for further evaluation and validation—all within 3 months. Further, AOAC has developed, and is currently finalizing, a validation study protocol, and the method is about to enter into collaborative study. AOAC validation of a method to detect PAHs in seafood is expected to take less than 6 months from start to finish.

In choosing a candidate method, AOAC reviewed approximately 30 methods for the detection of PAHs. Consequently, the PAH Working Group on Quantitative Methods, chaired by **Gina Ylitalo**, NOAA NWSFC, recommended a method by **Lucie Drabova et al.** at the Institute of Chemical Technology in Prague, Czech Republic as the most promising candidate method for further evaluation and, ultimately, validation as an AOAC-approved method.

In general, the method (Rapid Method for Simultaneous Determination of PAHs, Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers in Fish and Seafood Using GC-TOF/MS) is easy to perform, uses common laboratory equipment, and meets fitness-for-purpose and AOAC single-laboratory validation (SLV) requirements. The method uses a gas chromatography system coupled to a mass spectrometer detector that allows identification and quantification of all target PAHs.



AOAC INTERNATIONAL Collaborative Study

Final Protocol

Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Seafood using Gas Chromatography-Mass Spectrometry: A Collaborative Study

Katerina Mastovska
Covance Laboratories Inc

Wendy R. Sorenson
Covance Laboratories Inc

Jana Hajslova
Institute of Chemical Technology, Prague

Introduction

Within a European integrated project CONFIDENCE (Contaminants in food and feed: Inexpensive detection for control of exposure), Jana Hajslova's group at the Institute of Chemical Technology (ICT) in Prague, Czech Republic developed a method for the determination of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in fish and seafood using gas chromatography coupled with time-of-flight mass spectrometry (GC-TOFMS). This method was selected for further study as an AOAC collaborative study by the AOAC Stakeholders Panel on Seafood Contaminants (SPSC), which was formed as a response to the seafood contamination resulting from the recent oil spill in the Gulf of Mexico. The analytes for this collaborative study have been narrowed down to include only PAHs and some of the relevant PAH alkyl homologues. Having a rapid method is essential for quick determination of contaminants in food, especially after environmental disasters. The nineteen contaminants found in Table 1 will be studied in this collaborative study.

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Results for (shell)fish and fish feed

Example 2:

Heavy metals speciation: simplified method for inorganic arsenic

National Food Institute
Technical University of Denmark
DTU

Jens Sloth and Rie Rasmussen

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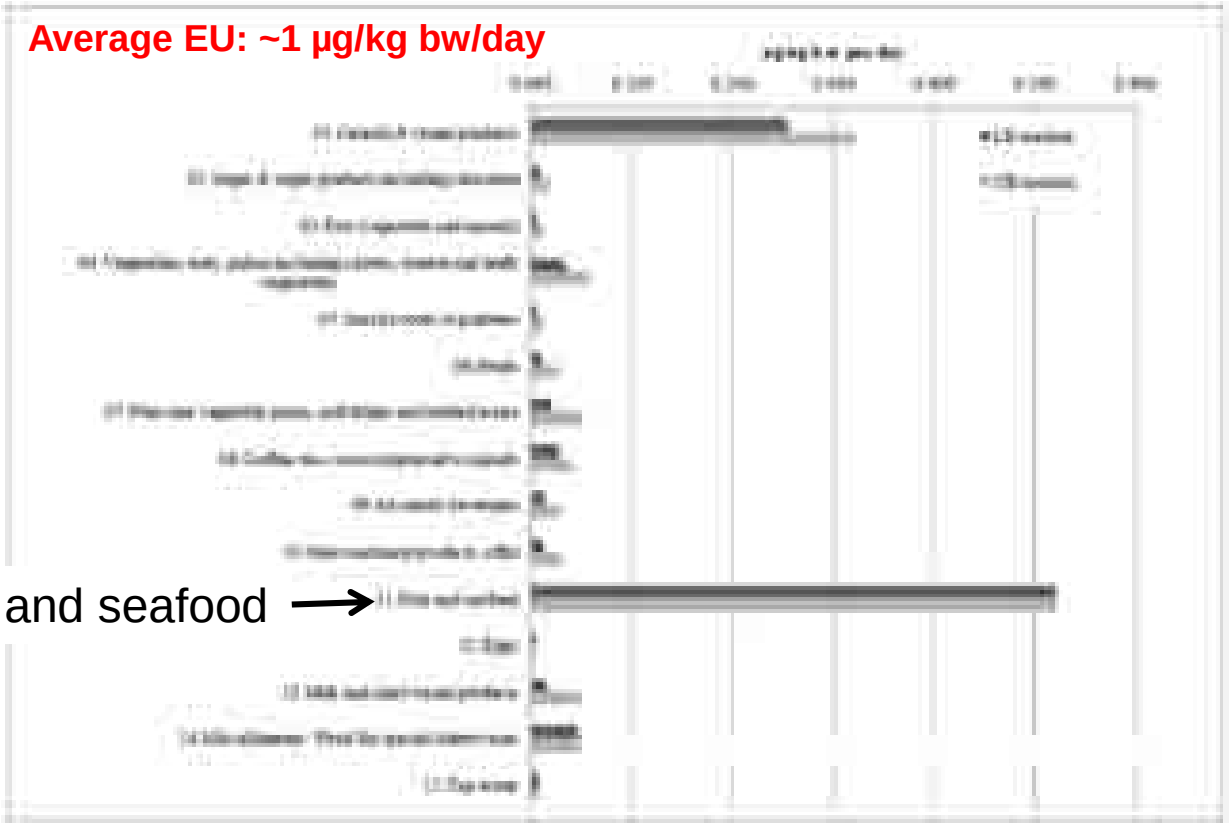
Selected sample types

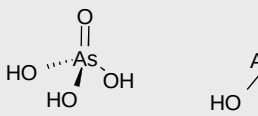
- Focus on seafood and marine feed

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

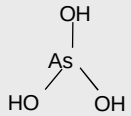


EFSA (2009), Scientific Opinion on Arsenic in Food

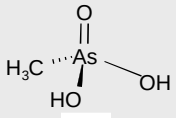
Arsenic compounds in the marine environment



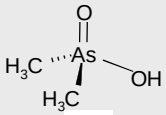
As(V)



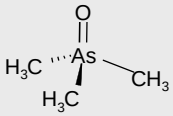
As(III)



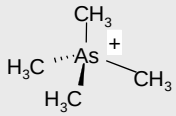
MA



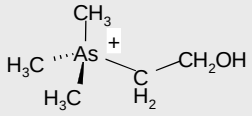
DMA



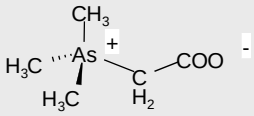
TMAO



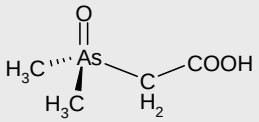
TETRA



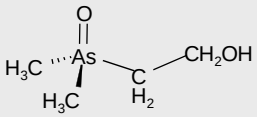
AC



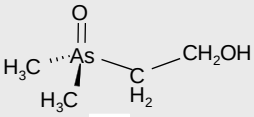
AB



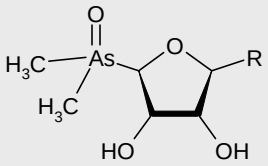
DMAA



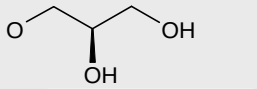
DMAE



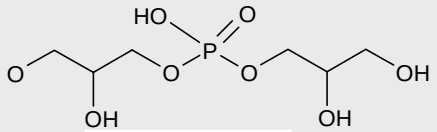
TMAP



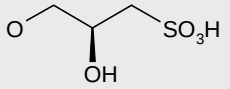
Dimethylarsinoylriboside



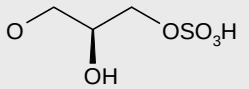
Dimethyl As-sugar 1



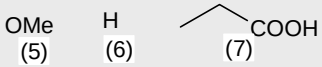
Dimethyl As-sugar 2

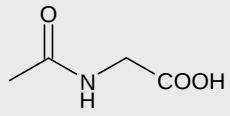


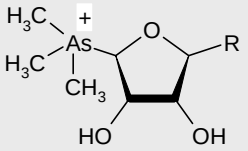
Dimethyl As-sugar 3



Dimethyl As-sugar 4







Trimethylarsonioriboside

R = 1,2,3,4,5

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

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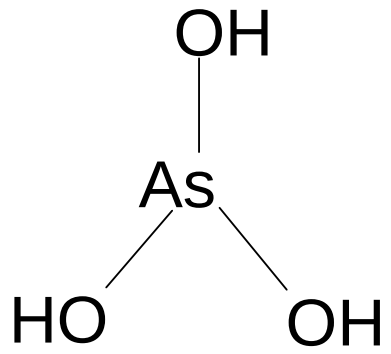
Arsenic compounds in the marine environment

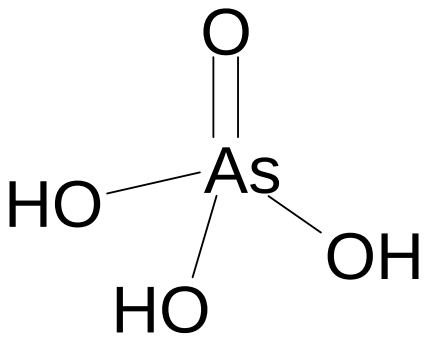
Arsenocosis

Most toxic form of arsenic!!

Inorganic arsenic



Arsenous acid
As(III)



Arsenic acid
As(V)

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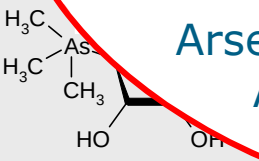

Trimethylarsonioriboside

Arsenic compounds in the marine environment

As(V)

As(III)

MA

DMA

TMAO

TETRA

AB

TMAP

Organoarsenic compounds - trimethylated species

Arsenobetaine
AB

Arsenocholine
AC

Trimethylarsoniopropionic acid
TMAP

Predominant form of arsenic in most seafood!!

Trimethylarsonioriboside

(8)

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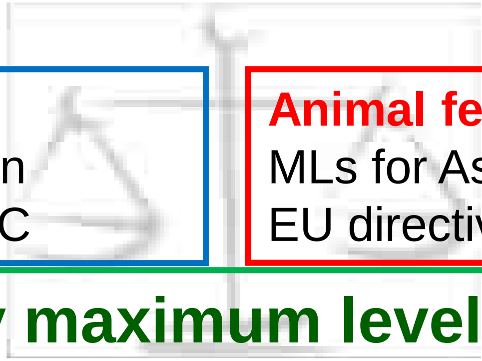
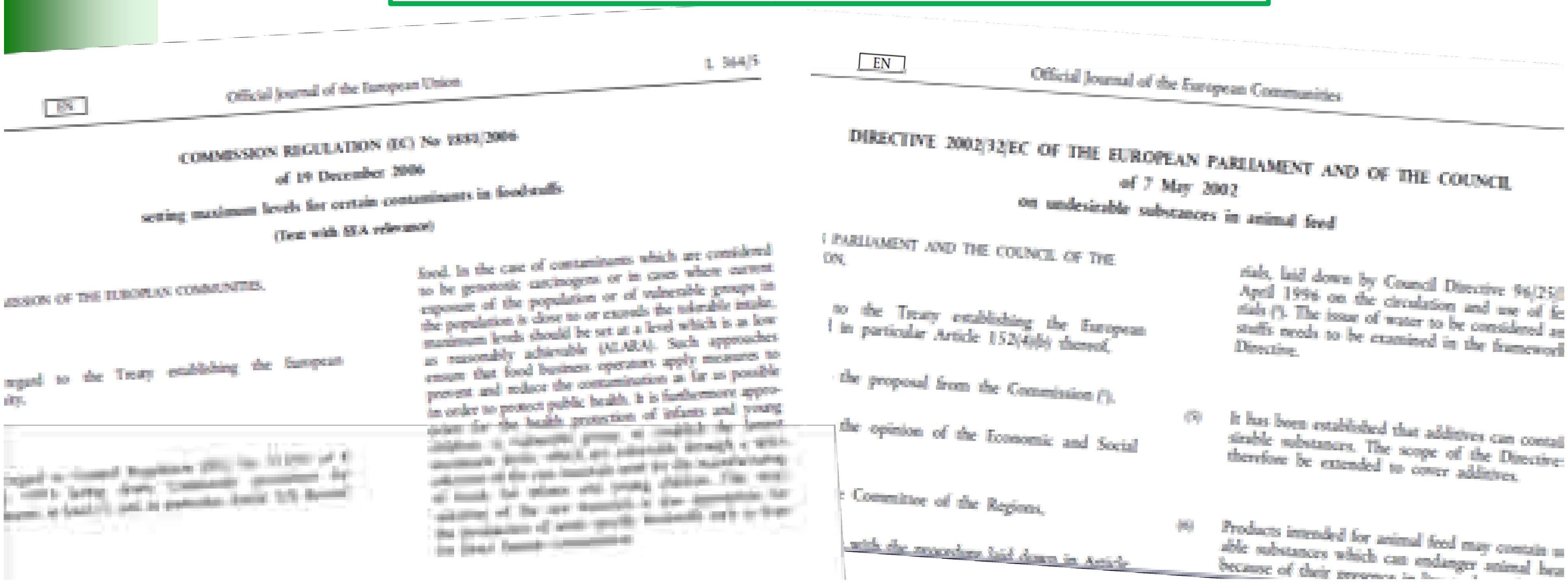
Current situation in EU legislation:



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
<u>total concentration</u> of the metals


EN
Official Journal of the European Union
L 364/5
COMMISSION REGULATION (EC) No 1881/2006
of 19 December 2006
setting maximum levels for certain contaminants in foodstuffs
(Text with EEA relevance)

EN
Official Journal of the European Communities
DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 7 May 2002
on undesirable substances in animal feed

EFSA (2009) opinion on arsenic in food

- **NEW!** $BMDL_{1.0}$ (bench mark dose) = 0.3 – 8 µg/kg bw per day for inorganic arsenic
- => EU dietary exposures within this range
- => Risk to some consumers cannot be excluded

- *"…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"*

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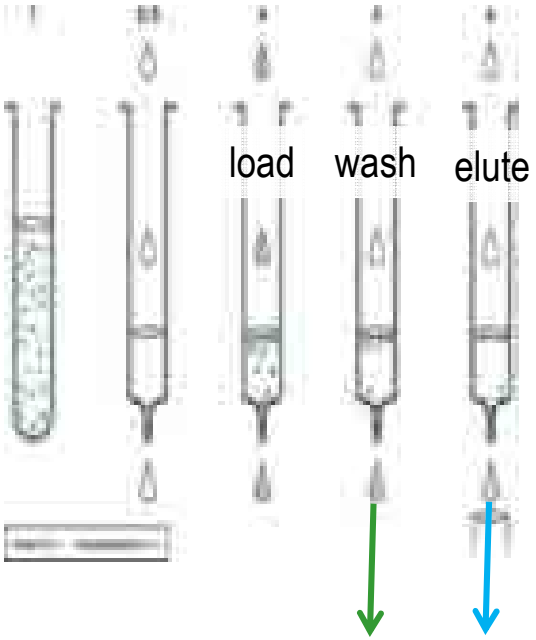
SPE-HG-AAS – a novel speciation alternative…

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

Inexpensive detection system



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

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National Food Institute Technical University of Denmark DTU

Results for (shell)fish and fish feed

Example 3:

Marine Biotoxins: high-throughput multiplex method for representatives from PSP / DSP / ASP shellfish toxin classes + Palytoxin (emerging): one test for all

Chris Elliott and Katrina Campbell

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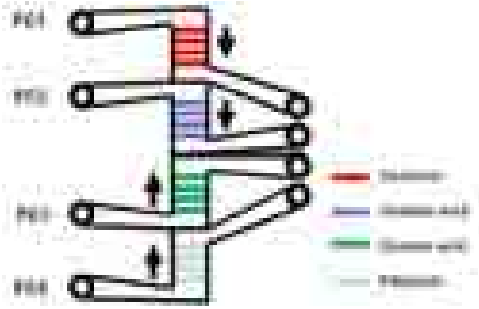
Results Marine Biotoxins

- Multiplex Immunoassay based on optical Surface Plasmon Resonance (SPR) biosensors



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Results for (shell)fish and fish feed

Example 4:

Mycotoxins in feed:

- Multi-dipstick methods
- Target toxins: DON, ZEA, FB_1, FB_2, T-2 and HT-2 toxins

Angelo Visconti and Veronica Lattanzio

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Mycotoxins: products and compounds

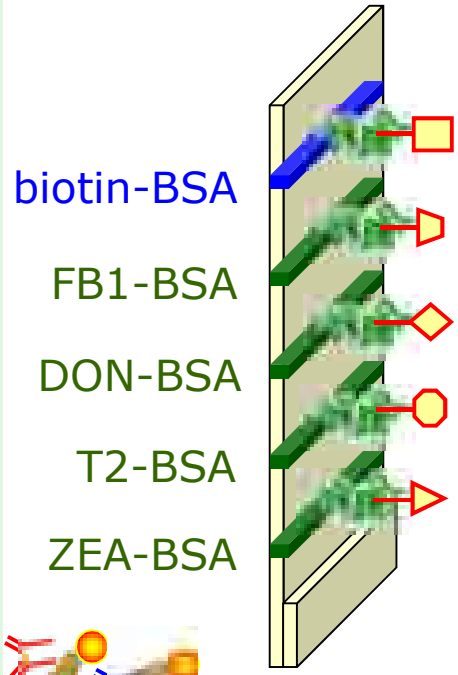
MAIZE

CORN GLUTEN FEED

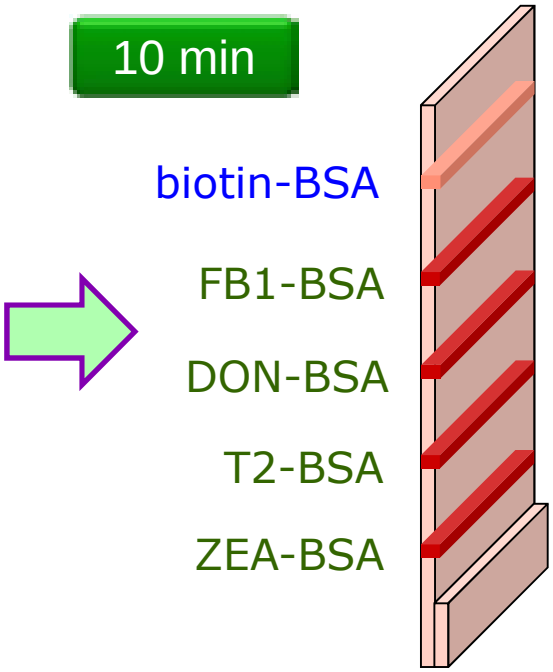
Target toxins: DON, ZEA, FB_1, FB_2, T-2 and HT-2 toxins

Mycotoxins: prototype multi-dipstick

- *Indirect competitive immunoassay; 10 min incubation at 40 °C*

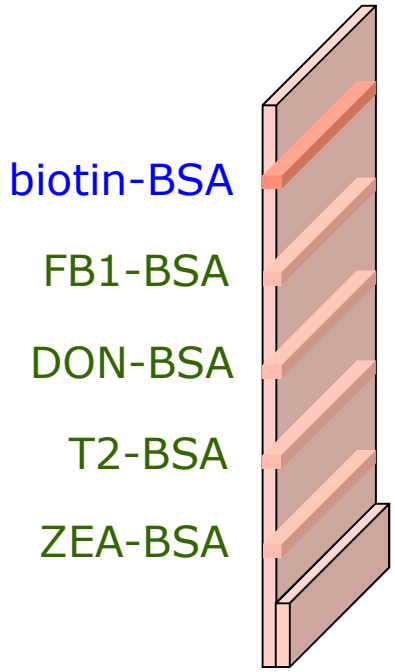


10 min



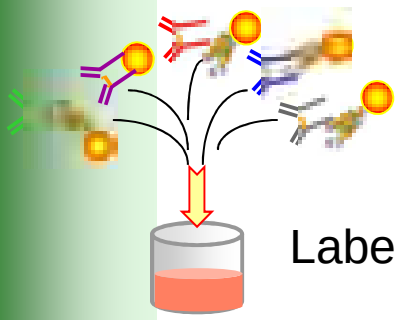
NEGATIVE sample

Test Lines darker than CTRL line



POSITIVE sample

Test Lines lighter than CTRL line



Labelled antibodies

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ISPA

unisensor Diagnostic Engineering

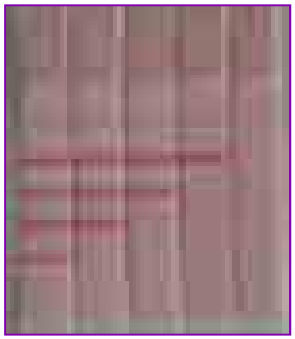
Mycotoxins: procedure for maize feed

Total analysis time: 30 min

- ✓ Add water; 2 min blending
- ✓ Add methanol; 2 min blending

Dilution and analysis

Incubation at 40 C, 10 min
Migration, 10 min



Negative sample
positive ZEA
Positive ZEA/T2
Positive ZEA/T2/DON
Positive ZEA/T2/DON/FB1

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Conclusions

- The CON*ff*IDENCE project contributes to improved safety of seafood and fish feed by the production of simplified and cost-effective tests for a.o.:
  - Persistent organic pollutants
  - Inorganic arsenic
  - Shellfish toxins
  - Mycotoxins
- Part of these tests (the dipsticks) can be used under “field” conditions, viz. in small laboratories; other tests require better equipped laboratories

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nutreco

nutrace

More information

Website: **www.conffidence.eu**

Contact:

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e-newsletter

(registration on website)

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Thank you for your attention !

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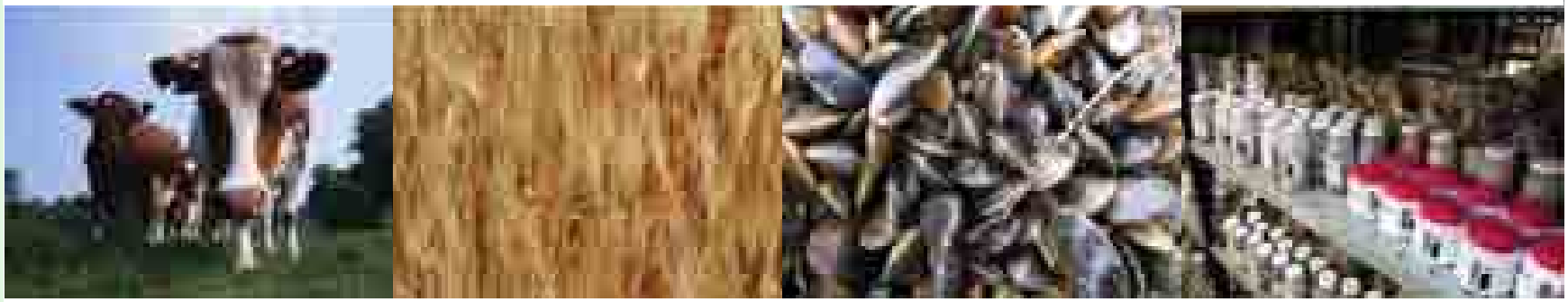

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