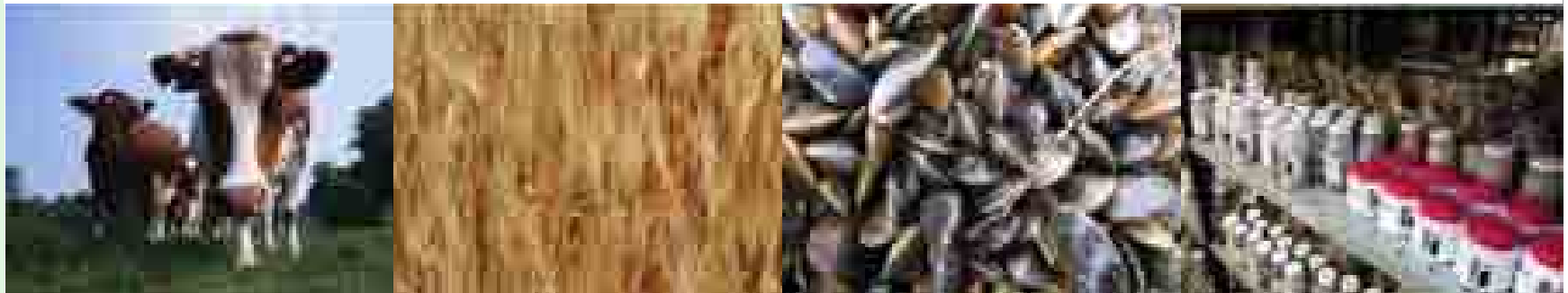


Rapid tests for chemical contaminants in seafood and fish feed – the CONffIDENCE research project (EU-FP7)

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

www.confidence.eu



Introduction (1)

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



Introduction (2)



Introduction (3)

The screenshot shows a BBC News article from Thursday, 7 September 2006. The main headline is "French oyster deaths investigated". The article reports that French authorities have launched an investigation following the death of two people who had eaten oysters in the town of Arcachon, south-west France. The two victims, aged 66 and 77, died in separate incidents during the week. The article notes that so far, no link has been established between the deaths and eating the shellfish, according to authorities. It mentions that concern was raised because of the ban on the sale and consumption of Arcachon bay oysters imposed in August after tests revealed a high level of toxins. A quote from a source states: "It is the third oyster ban in the last 18 months in the region of Arcachon, which is famous for its oysters - although it is not clear where the toxins come from." The article concludes that this has angered local farmers, who say the ban is affecting their livelihood.

French oyster deaths investigated

The French authorities have launched an investigation following the death of two people who had eaten oysters in the town of Arcachon, south-west France.

The two - aged 66 and 77 - died in separate incidents during the week.

So far, no link has been established between the deaths and eating the shellfish, authorities say.

Concern was raised because of the ban on the sale and consumption of Arcachon bay oysters imposed in August after tests revealed a high level of toxins.

It is the third oyster ban in the last 18 months in the region of Arcachon, which is famous for its oysters - although it is not clear where the toxins come from.

This has angered local farmers, who say the ban is affecting their livelihood.

UK: We should not jump to conclusions

French south-western



Introduction (4)

- Globalisation of trade in food and feed ingredients



Introduction (5)

What is the challenge ?

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



CONFIDENCE in a nutshell

CONTaminants in *food* and *feed*:
Inexpensive DETection
for Control of Exposure



CONFIDENCE 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)



The commodities

Food

&

Feed

➤ **Fish/shellfish**

Fish feed

➤ Cereals

Cereal-based feed

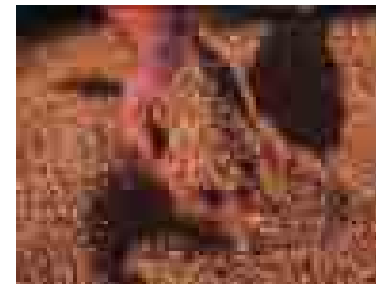
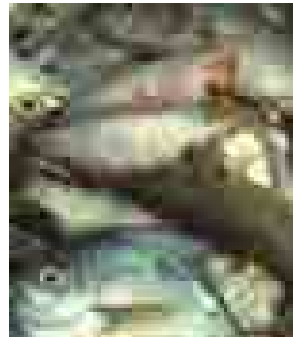
➤ Potatoes/vegetables

➤ Honey

➤ Eggs

➤ Meat

➤ Dairy products



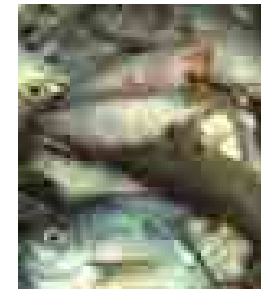
The target contaminants

- 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

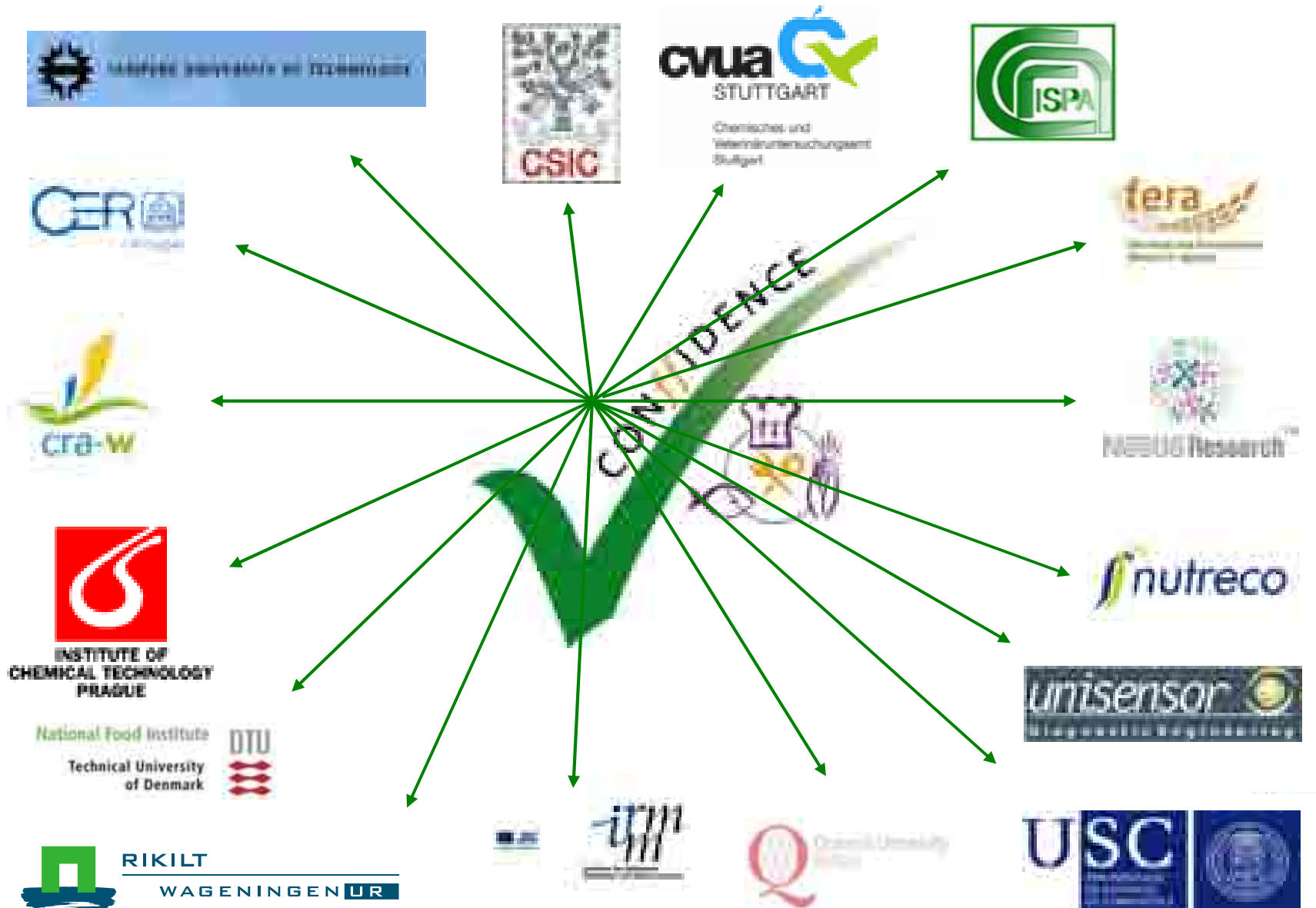


Target contaminants for fish (feed)

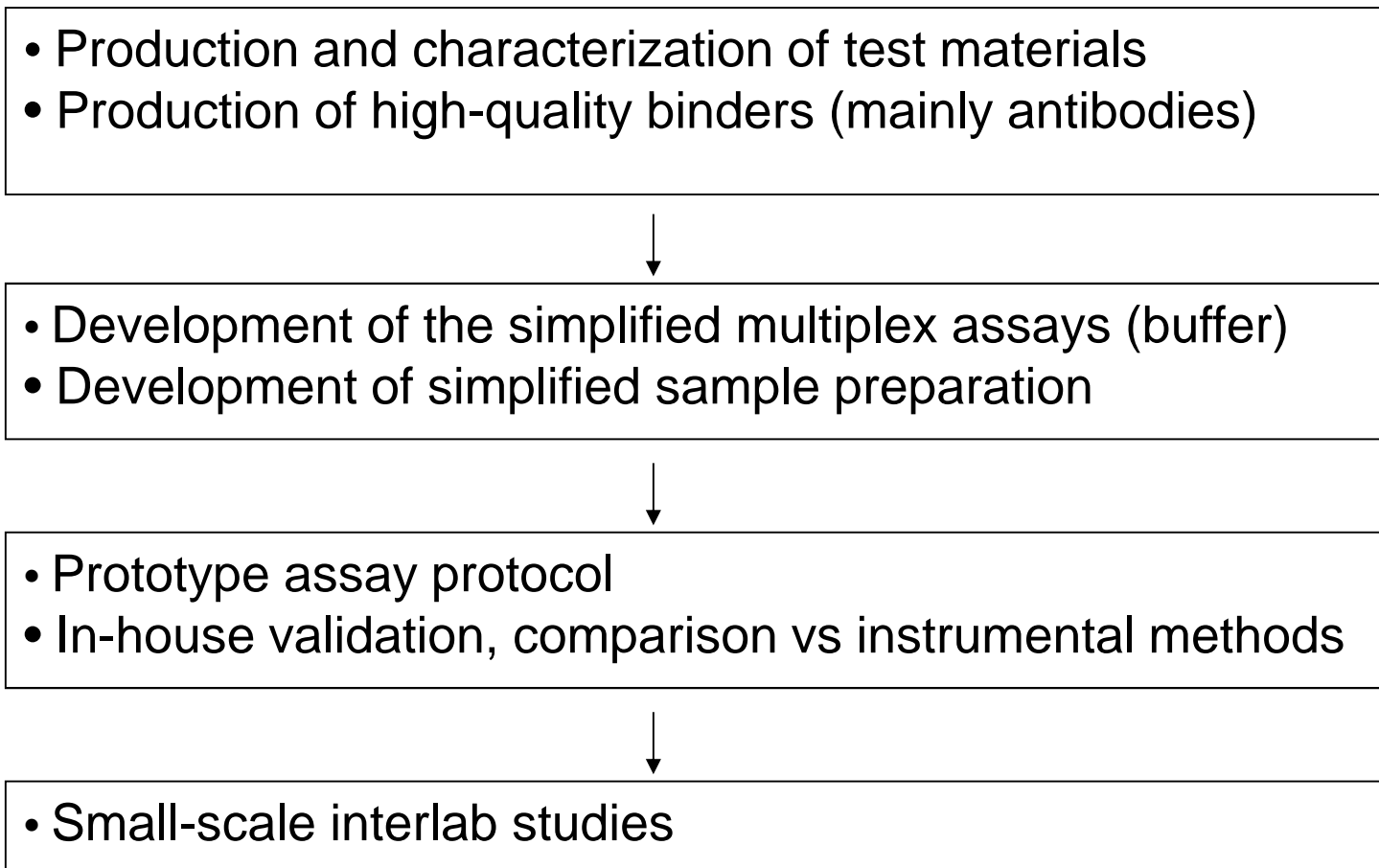
- POPs:
 - dioxin-like PCBs
 - brominated flame retardants
 - polycyclic aromatic hydrocarbons (PAH)
- Perfluorinated compounds (PFCs)
- Veterinary drugs: - antibiotics
- Heavy metals speciation: inorganic arsenic, methyl mercury
- Biotoxins:
 - marine biotoxins
 - mycotoxins (cereals)



The consortium



Current state of progress



Year 1

Year 4



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



INSTITUTE OF
CHEMICAL TECHNOLOGY
PRAGUE

Jana Hajslova and Jana Pulkrabova



Integrated sample preparation

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

Extraction
Shaking (H₂O + ethylacetate)

Isolation
10 min

Partition (transfer into organic phase)
induced by MgSO₄ + NaCl

Clean up
30 min

Clean up
Silica minicolumn

Identification & quantification
1 h

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

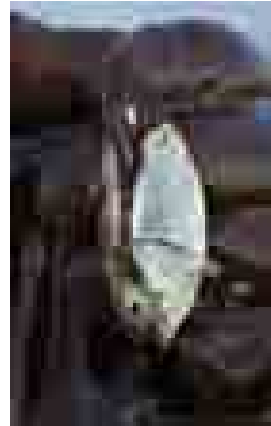
**6 SAMPLES /
< 1 HOUR**



**SINGLE GC
INJECTION**



Oil Spill - Gulf Of Mexico 2010



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.**



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





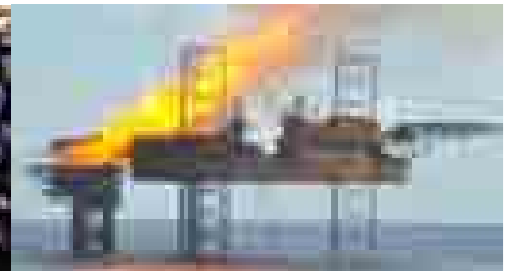
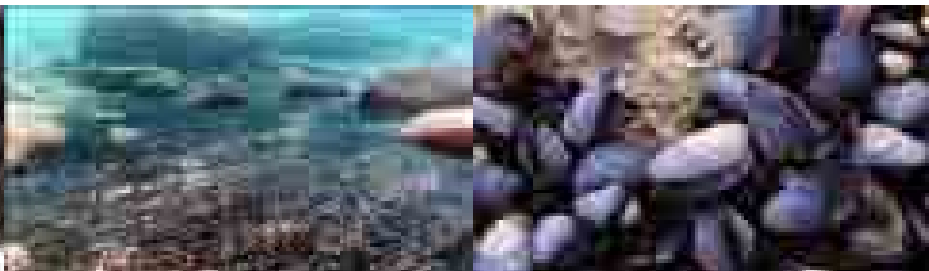
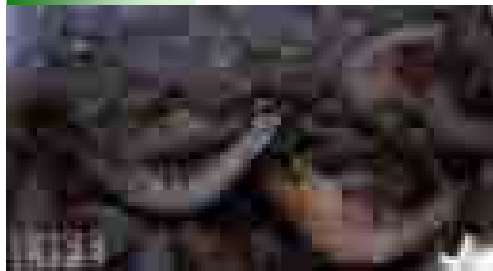
NEWS FLASH

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 Mastovcova
Covance Laboratories Inc

Wendy R. Sorenson
Covance Laboratories Inc

Jana Hajdlova
Institute of Chemical Technology, Prague

Introduction

Within a European integrated project CONFIDENCE (Contaminants in food and feed: Inexpensive detection for control of exposure), Jana Hajdlova'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.

Results for (shell)fish and fish feed

Example 2:

Heavy metals speciation: simplified method for
inorganic arsenic

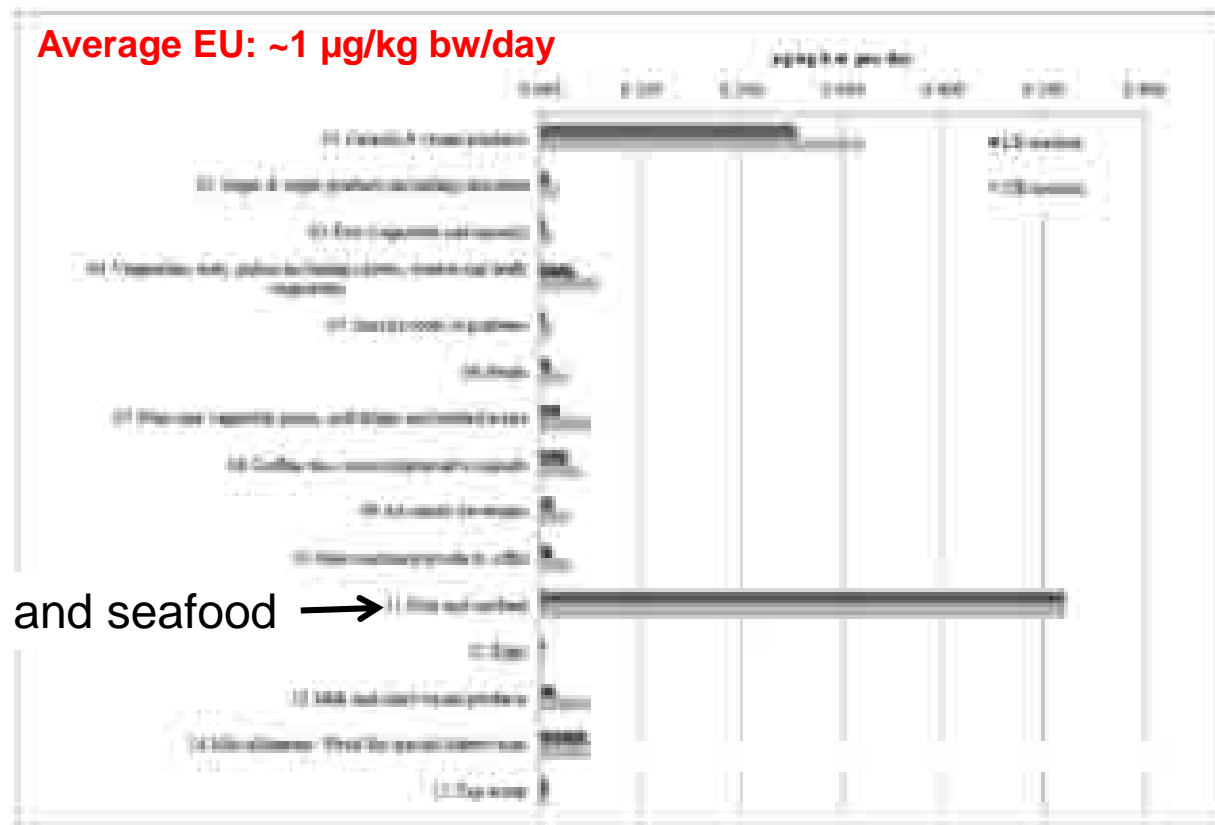


Jens Sloth and Rie Rasmussen



Selected sample types

- Focus on seafood and marine feed
- Seafood is the main dietary source of **arsenic**



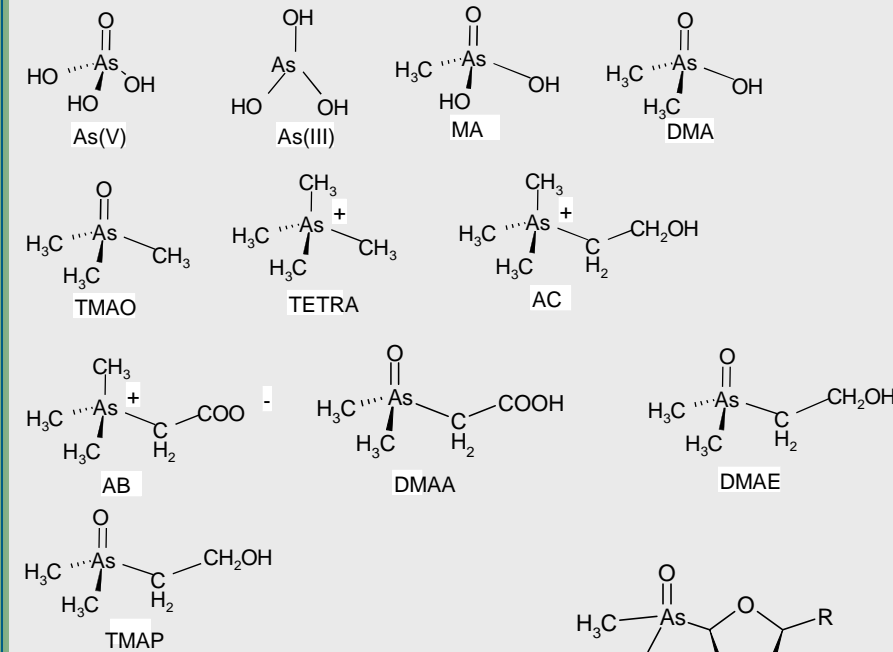
Fish and seafood →



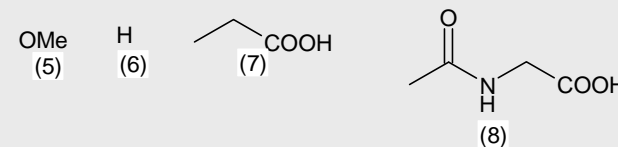
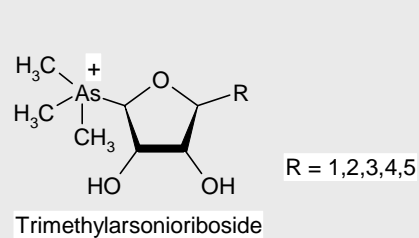
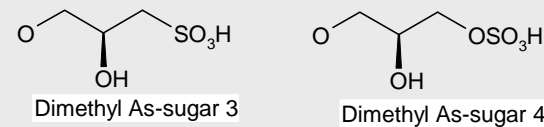
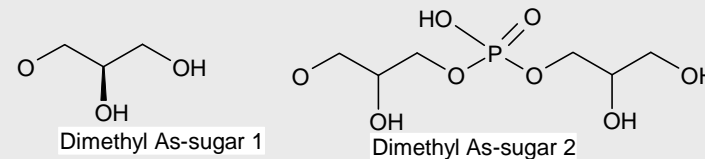
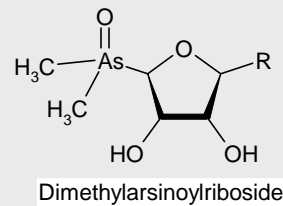
EFSA (2009), *Scientific Opinion on Arsenic in Food*



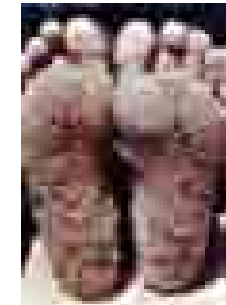
Arsenic compounds in the marine environment



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



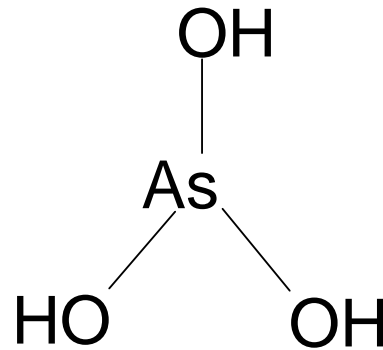
Arsenic compounds in the marine environment



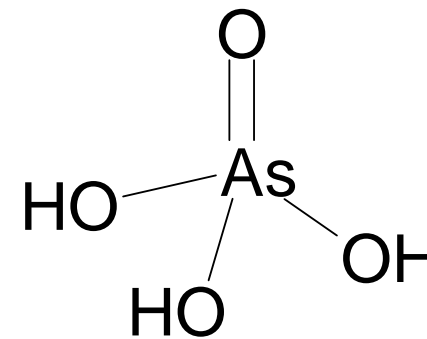
Arsenocosis

Most toxic form of arsenic!!

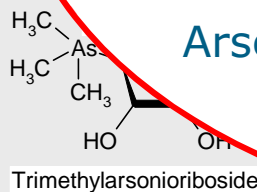
Inorganic arsenic



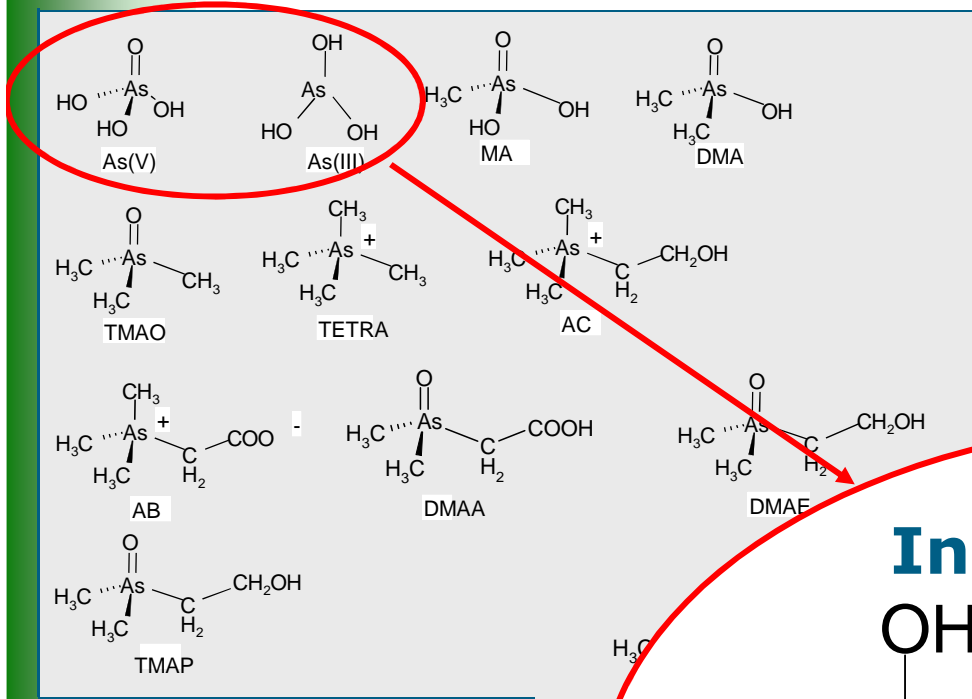
Arsenous acid
As(III)



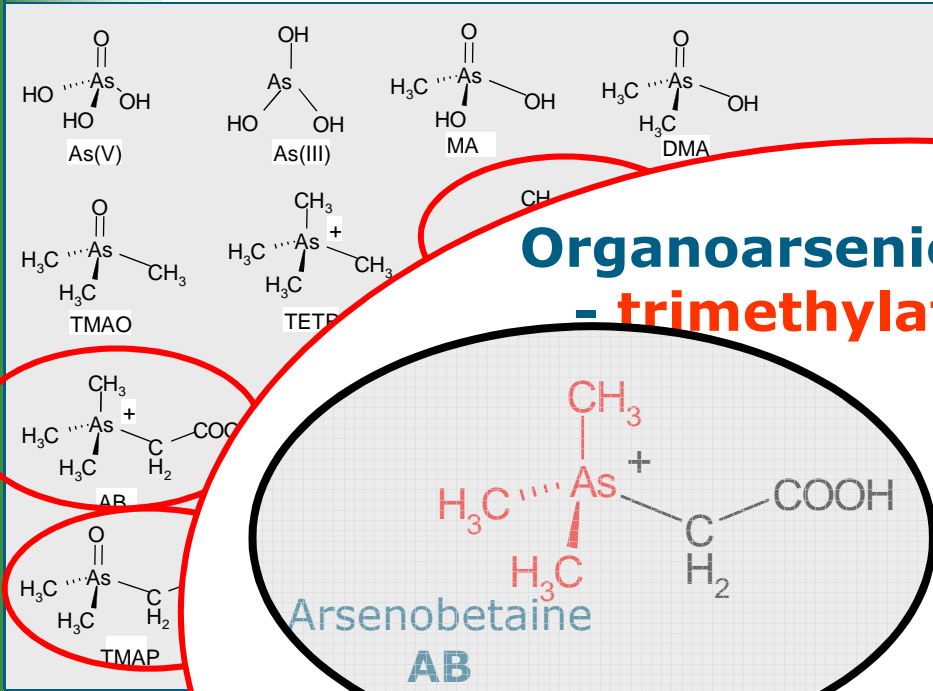
Arsenic acid
As(V)



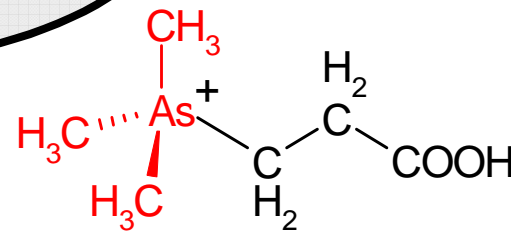
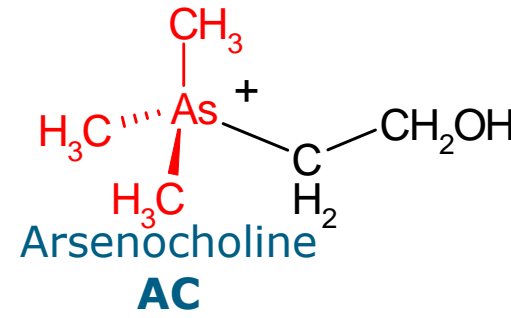
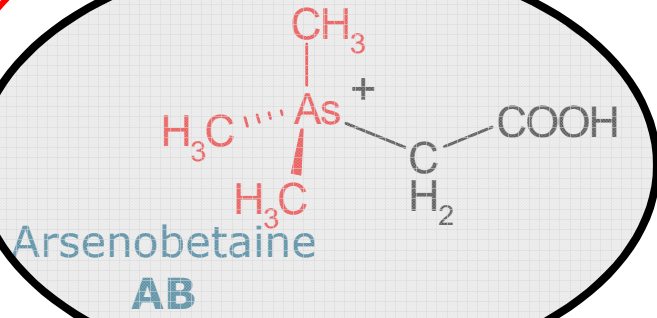
Trimethylarsonioriboside



Arsenic compounds in the marine environment



Organoarsenic compounds - trimethylated species



Predominant form of arsenic in most seafood!!

Trimethylarsoniopropionic acid
TMAP

Trimethylarsonioriboside

Current situation in EU legislation:

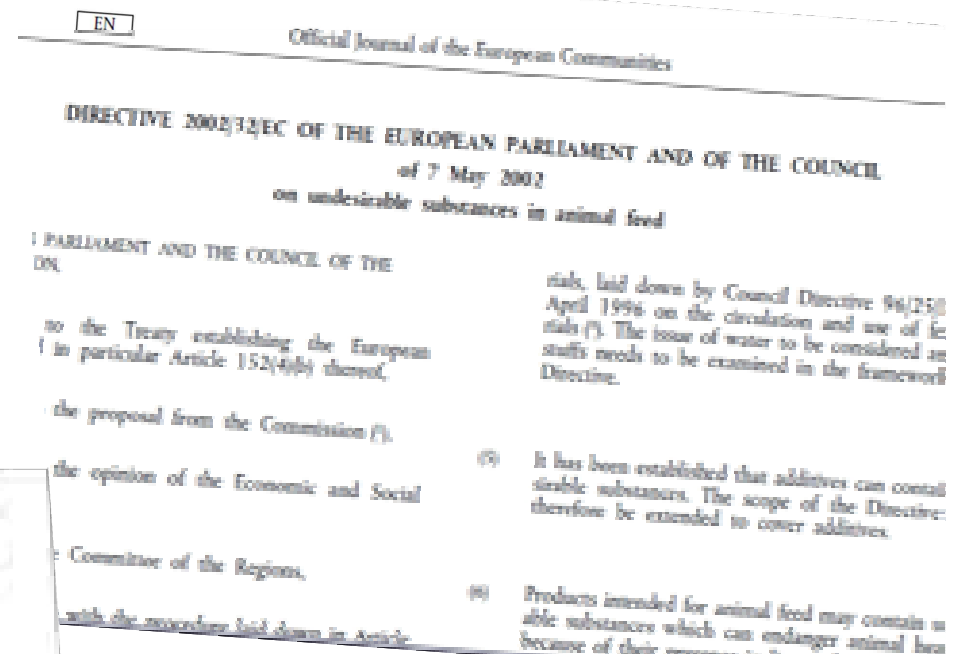
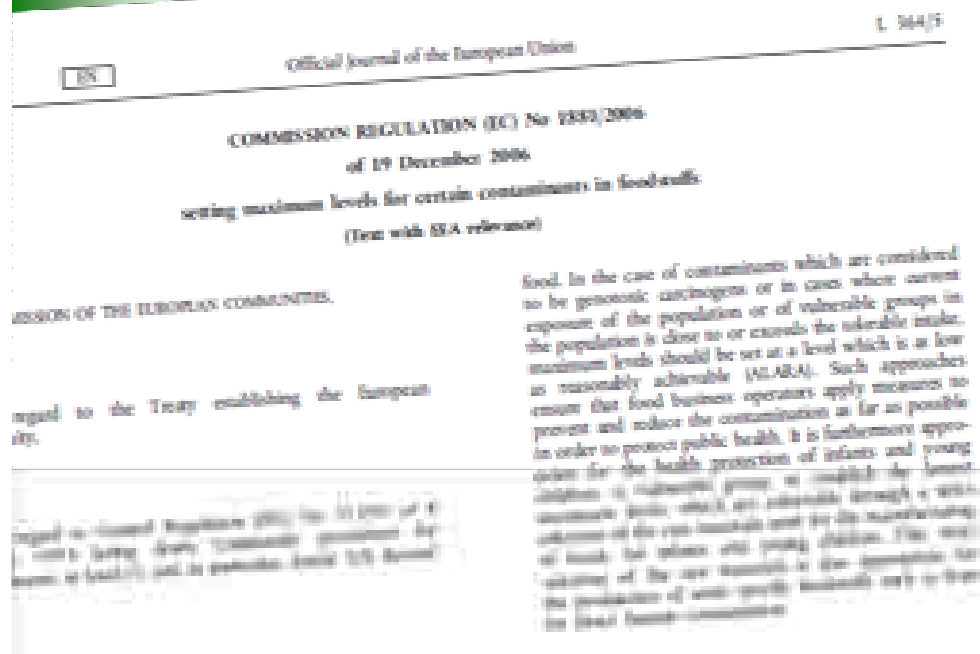
Foodstuffs

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**



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”



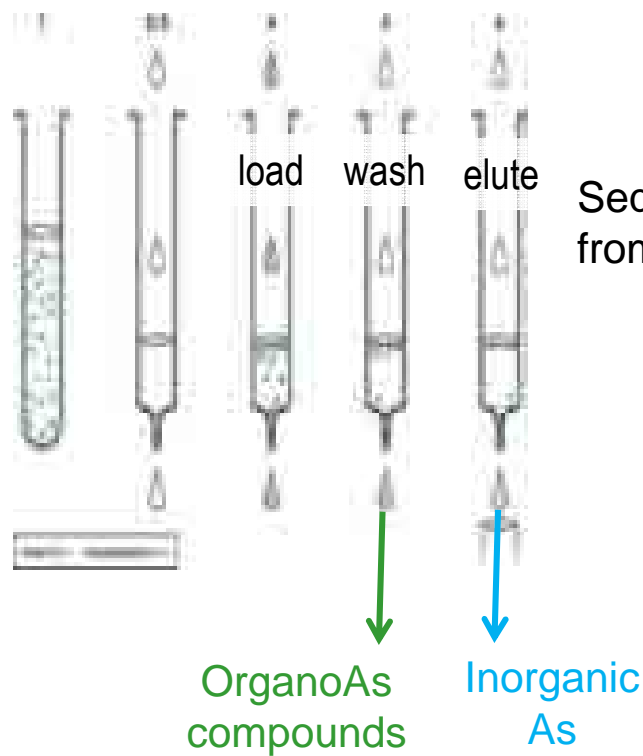
SPE-HG-AAS – a novel speciation alternative...

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



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

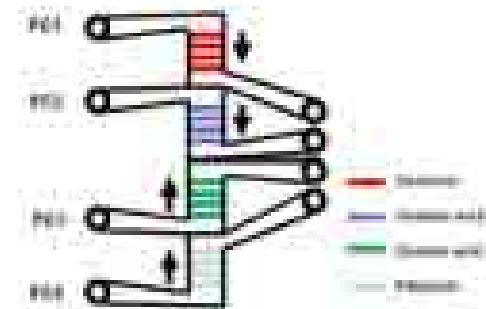


Chris Elliott and Katrina Campbell



Results Marine Biotoxins

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



Results for (shell)fish and fish feed

Example 4:

Mycotoxins in feed:

- Multi-dipstick methods
- Target toxins: **DON**, **ZEA**, **FB₁**, **FB₂**, **T-2** and **HT-2** toxins



Angelo Visconti and Veronica Lattanzio



Mycotoxins: products and compounds



MAIZE

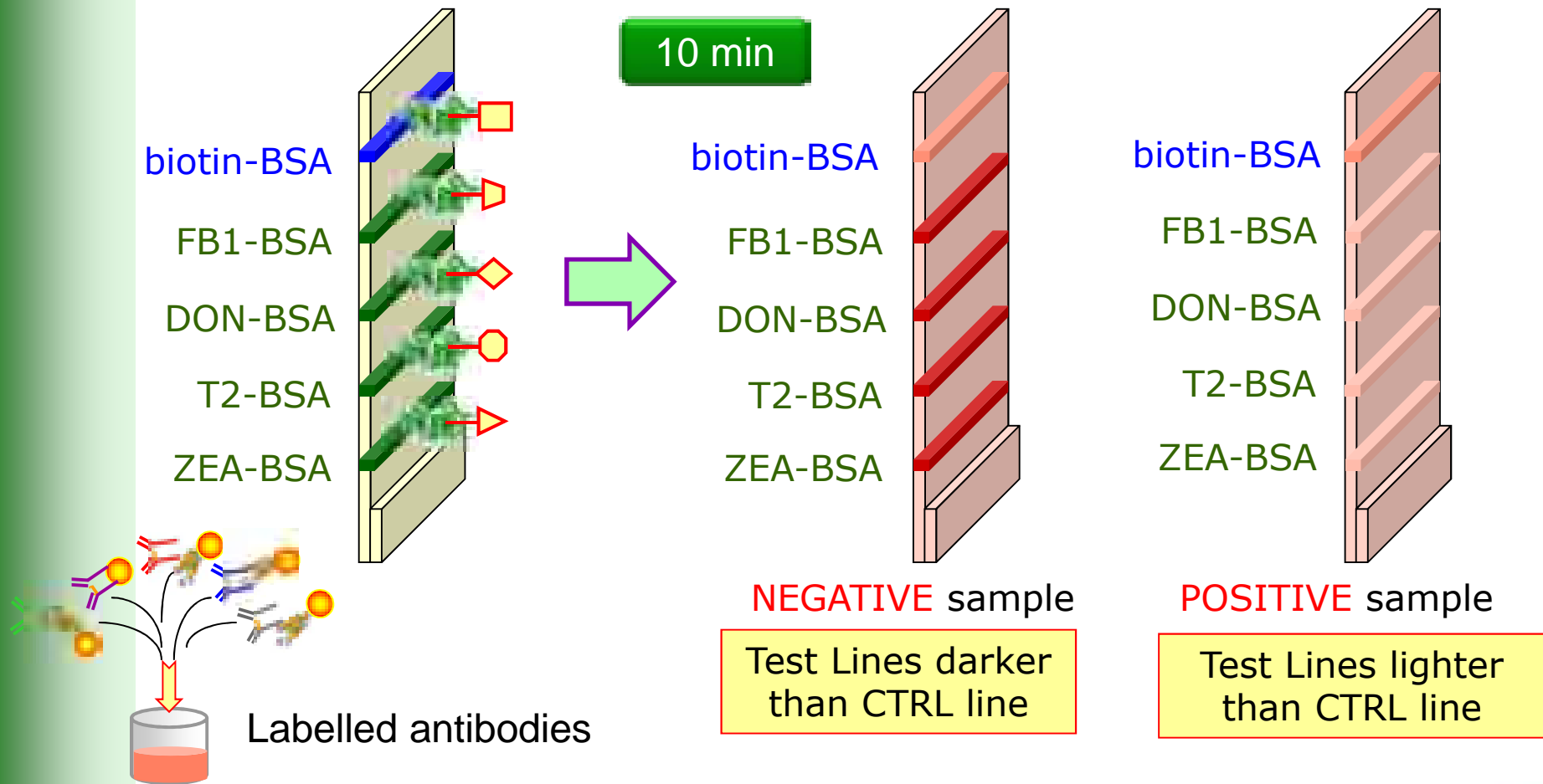


CORN GLUTEN FEED

Target toxins: **DON**, **ZEA**, **FB₁**, **FB₂**, **T-2** and **HT-2** toxins

Mycotoxins: prototype multi-dipstick

➤ Indirect competitive immunoassay; 10 min incubation at 40 °C



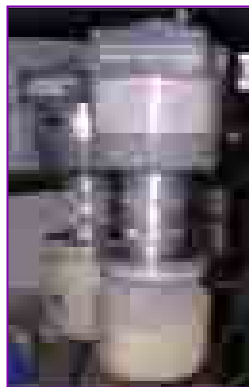
Mycotoxins: procedure for maize feed



**Total analysis
time: 30 min**



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



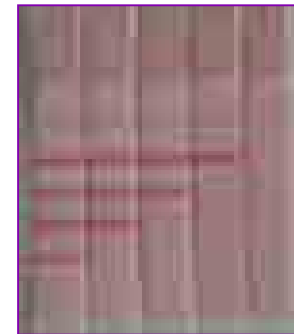
Dilution and
analysis



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



Conclusions

- The CONFIDENCE 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



More information

Website: www.confidence.eu

Contact:

coordination@confidence.eu

e-newsletter

(registration on website)



Acknowledgements

- The CONfidence project has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n°KBBE-211326
- Many colleagues from CONfidence partners



Thank you for your attention !

www.confidence.eu

