Ensuring food safety through effective monitoring of chemical contaminants:

CON*ff*IDENCE results and future challenges

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SEVENTH FRAMEWORK PROGRAMME

CONffIDENCE

Welcome to the final Stakeholder Workshop !

CON*ff*IDENCE in a nutshell

Chemical CONtaminants

CON*ff*IDENCE in a nutshell

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

Inexpensive DEtectioN

for Control of Exposure

The objectives

- Development and validation of new simplified inexpensive detection methods for chemical contaminants from farm to fork
- Improved exposure assessment through monitoring of selected contaminants
- Contribute to validation of predictive hazard behaviour models
- Dissemination and training of new detection methods to all relevant stakeholders, to advance technology exploitation

CONffIDENCE passport

- FP7 Collaborative Project first call “Food, Agriculture & Fisheries, and Biotechnology”
- Duration: May 2008 – December 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)

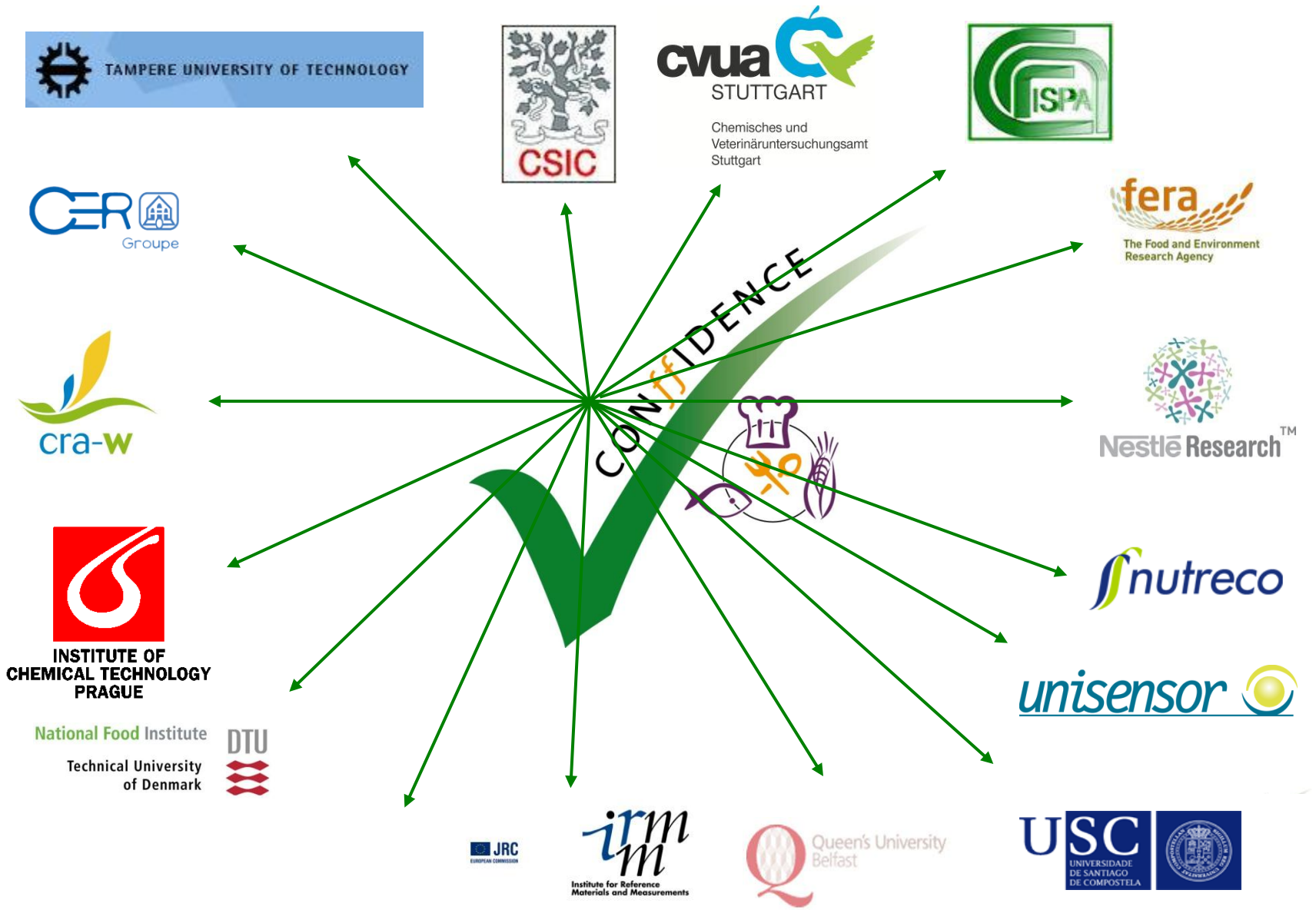
The commodities

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

The target contaminants

- ✓ Organic pollutants (WP 1 cluster)
  - POPs (Persistent Organic Pollutants)
  - Perfluorinated compounds
  - Pesticides
- ✓ Veterinary drugs (WP 2 cluster)
  - Antibiotics
  - Coccidiostats
- ✓ Heavy metals (WP 3)
- ✓ Biotoxins (WP 4 cluster):
  - Alkaloids
  - Marine biotoxins
  - Mycotoxins

The consortium



Work package 1a & 1b & 1c cluster

Organic pollutants: POPs, Perfluorinated compounds & Pesticides

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Work package WP1a

Persistent organic pollutants (POPs)

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Achievement 1: Integrated sample preparation

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

Isolation
10 min

Extraction
Shaking (H_2O + ethyl acetate)

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

Clean up
30 min

Clean up
Silica SPE minicolumn

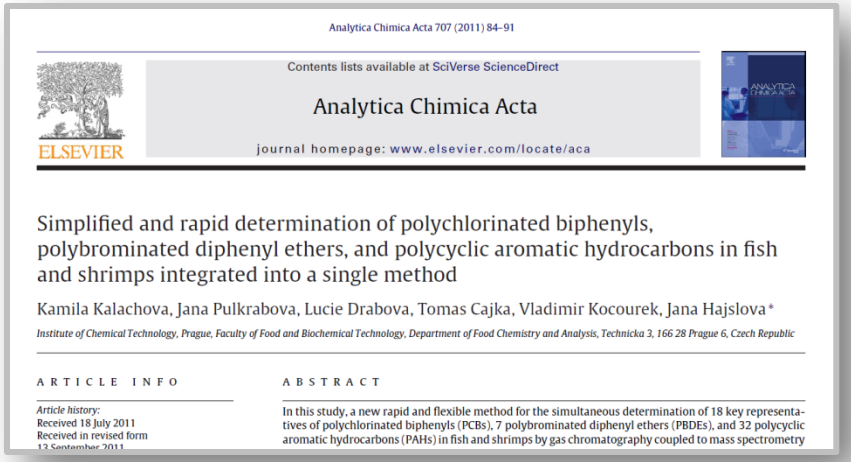
Identification & quantification
1 h

Identification & quantification
GC×GC–TOFMS (EI)
GC–MS/MS (EI)

TIME SAVING
- One sample = < 1hour
- Six samples at one time

SOLVENT VOLUME REDUCTION

COST/LABOUR SAVING



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ELSEVIER

Simplified and rapid determination of polychlorinated biphenyls, polybrominated diphenyl ethers, and polycyclic aromatic hydrocarbons in fish and shrimps integrated into a single method

Kamila Kalachova, Jana Pulkrabova, Lucie Drabova, Tomas Cajka, Vladimir Kocourek, Jana Hajslova*

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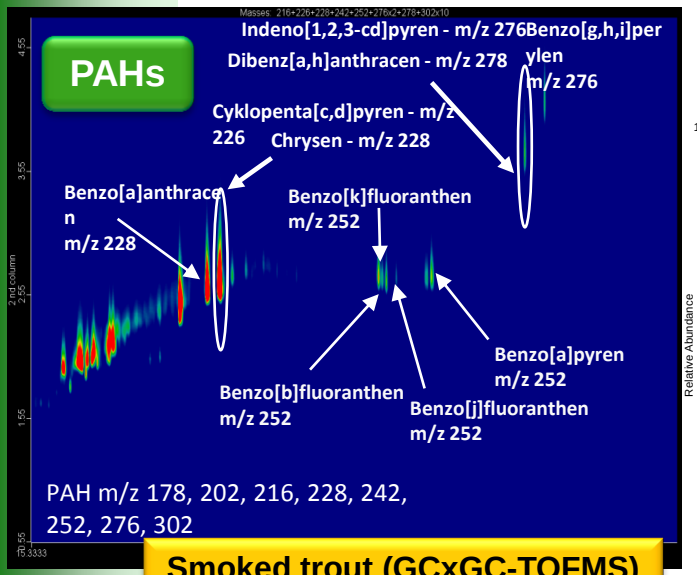
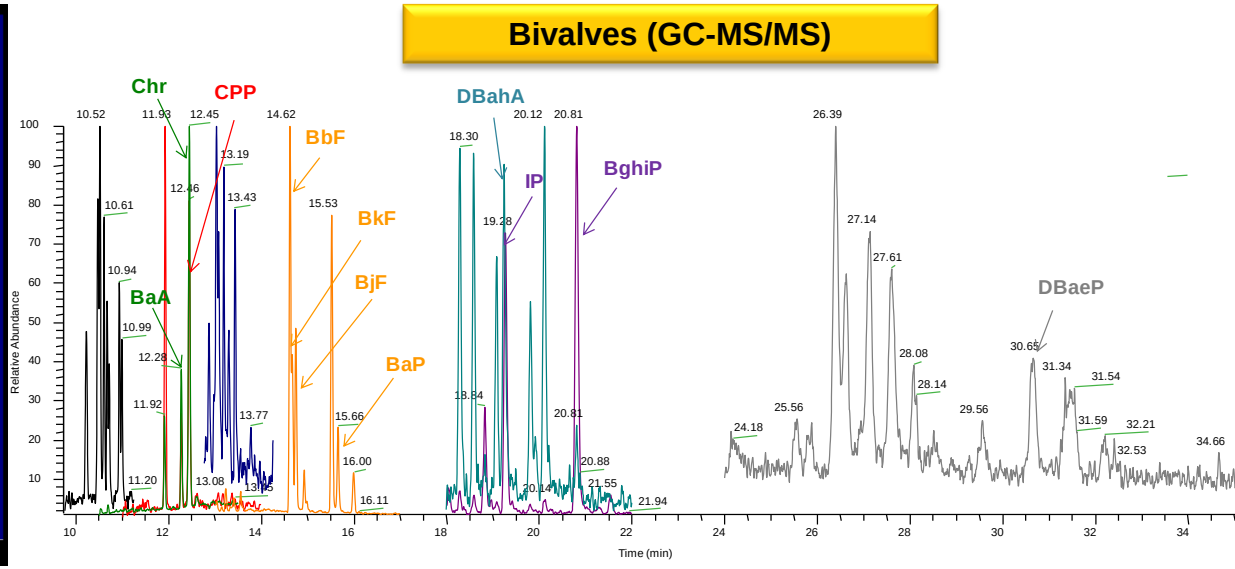
ABSTRACT

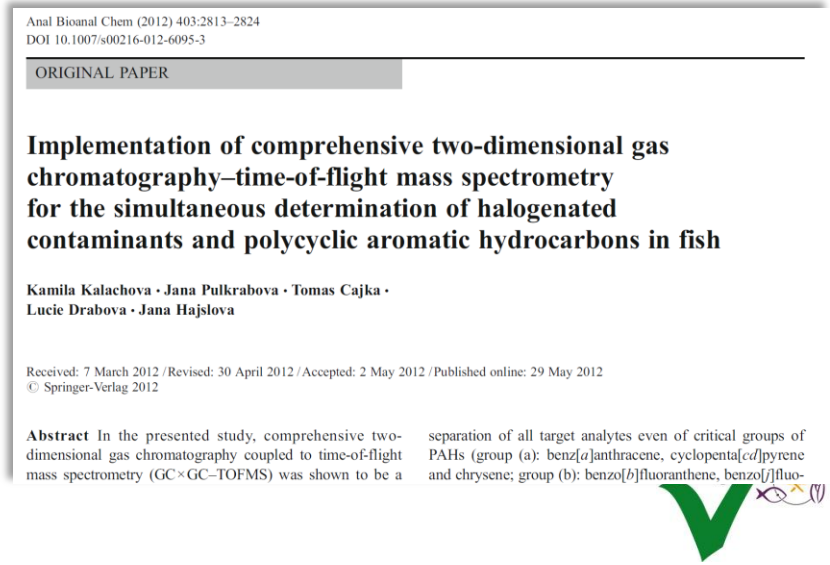
In this study, a new rapid and flexible method for the simultaneous determination of 18 key representatives of polychlorinated biphenyls (PCBs), 7 polybrominated diphenyl ethers (PBDEs), and 32 polycyclic aromatic hydrocarbons (PAHs) in fish and shrimps by gas chromatography coupled to mass spectrometry

Achievement 2: GC–MS methods





Parameter / feature	GCxGC-TOFMS	GC-MS/MS
Chromatographic resolution	+++	+
Selectivity of detection	++ (deconvolution)	++ (products ions)
Detection limits	+	+++
Data handling (time demands)	-	+
Retrospective data mining	+	-
Availability in common control labs	-	++



Anal Bioanal Chem (2012) 403:2813–2824
DOI 10.1007/s00216-012-6095-3

ORIGINAL PAPER

Implementation of comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the simultaneous determination of halogenated contaminants and polycyclic aromatic hydrocarbons in fish

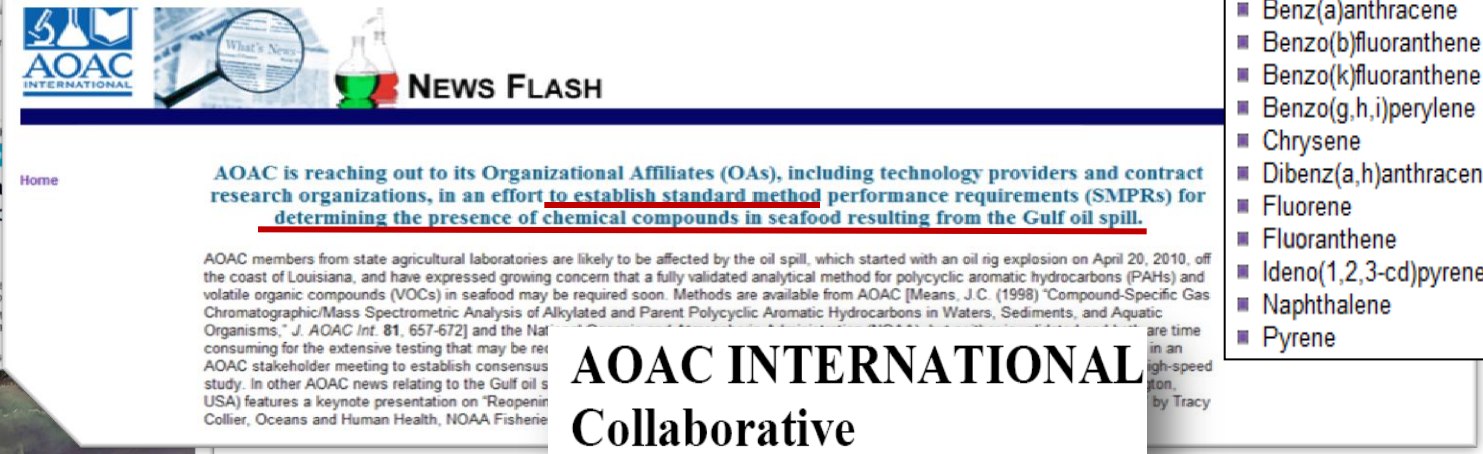
Kamila Kalachova · Jana Pulkrabova · Tomas Cajka · Lucie Drabova · Jana Hajslova

Received: 7 March 2012 / Revised: 30 April 2012 / Accepted: 2 May 2012 / Published online: 29 May 2012

Abstract In the presented study, comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC–TOFMS) was shown to be a separation of all target analytes even of critical groups of PAHs (group (a): benz[*a*]anthracene, cyclopenta[*cd*]pyrene and chrysene; group (b): benzo[*b*]fluoranthene, benzo[*j*]fluo-

AOAC accepts and validates CONffIDENCE method

- Anthracene/phenanthrene
- Benz(a)anthracene
- Benzo(b)fluoranthene
- Benzo(k)fluoranthene
- Benzo(g,h,i)perylene
- Chrysene
- Dibenz(a,h)anthracene
- Fluorene
- Fluoranthene
- Ideno(1,2,3-cd)pyrene
- Naphthalene
- Pyrene



AOAC INTERNATIONAL Collaborative Study

Final Protocol

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 th allows identification and quantification of all target PAHs.

Hydrocarbons (PAHs) in Seafood using Gas
A Collaborative Study

Covance Laboratories Inc.

Wendy R. Sorenson
Covance Laboratories Inc.

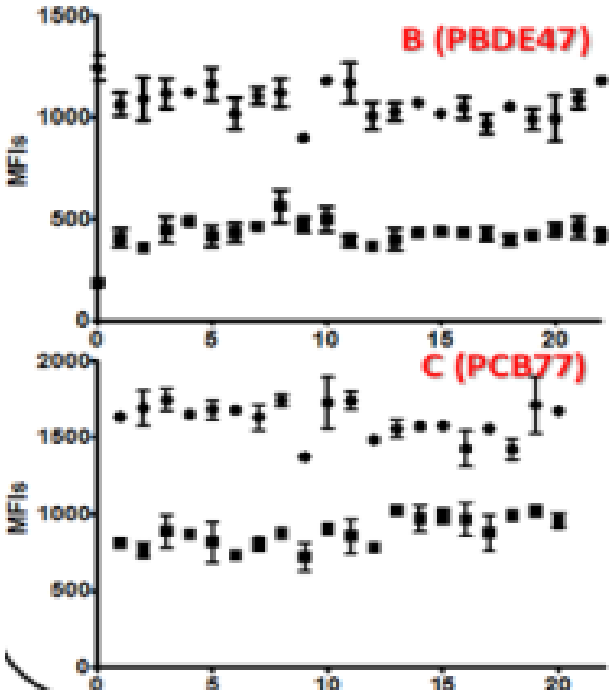
Jana Hajslova
Institute of Chemical Technology, Prague

Introduction

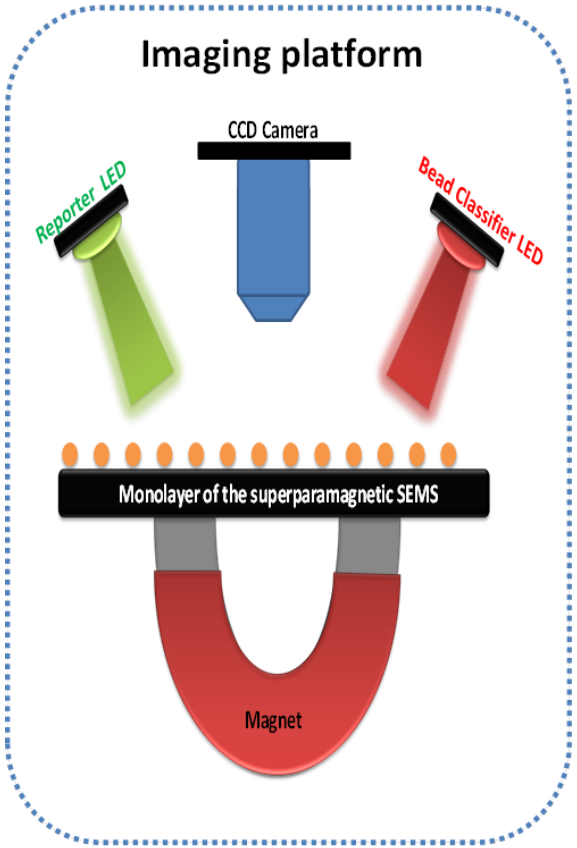
Within a European integrated project CONffIDENCE (Contaminants in food and feed: Inexpensive detection for control of exposure), Jana Hajslova's group at the Institute of Chemical Technology (ICT) in Prague, Czech Republic developed a method for the determination of polycyclic aromatic hydrocarbons (PAHs), 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.

Achievement 3: screening POPs in fish

- Multiple persistent pollutants screened simultaneously
- Demonstrated for lean fish



MAGPIX



- High throughput 96 wells assay
- Imaging bead based assay

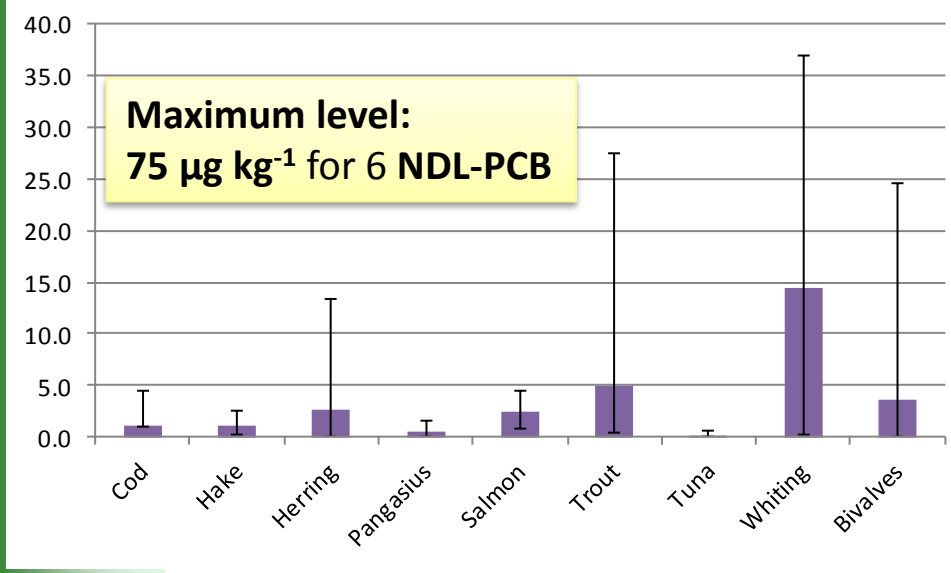
Achievement 4: monitoring survey

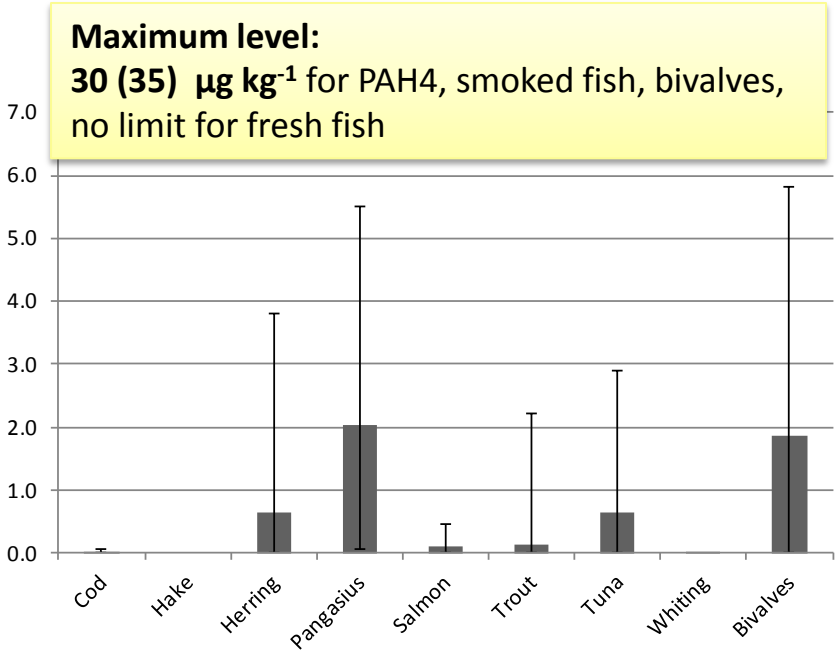
- Analysis of batch of ca. 150 samples, based on EU-MSFD-descriptor 9
- Co-occurrence of POPs & PUFA, PFAS, heavy metals, for risk-benefit discussions
- Data to be provided to **EFSA**

Species	Region	Lead for collection of samples
Herring	Baltic sea	DTU
	North sea	RIKILT
	Atlantic ocean	DTU
Cod/whiting/hake	North sea	ICT
	Atlantic ocean	ICT
	Mediterranean sea	CSIC
Trout and salmon	Czech Republic	ICT
	Spain	CSIC
	Scandinavia	DTU
Bivalves	Scandinavia	DTU
	The Netherlands	RIKILT
	Mediterranean Sea	CSIC
Tuna	Canned, in water, preferably from Europe	All
Pangasius	Mostly Vietnam	All

efsa
European Food Safety Authority

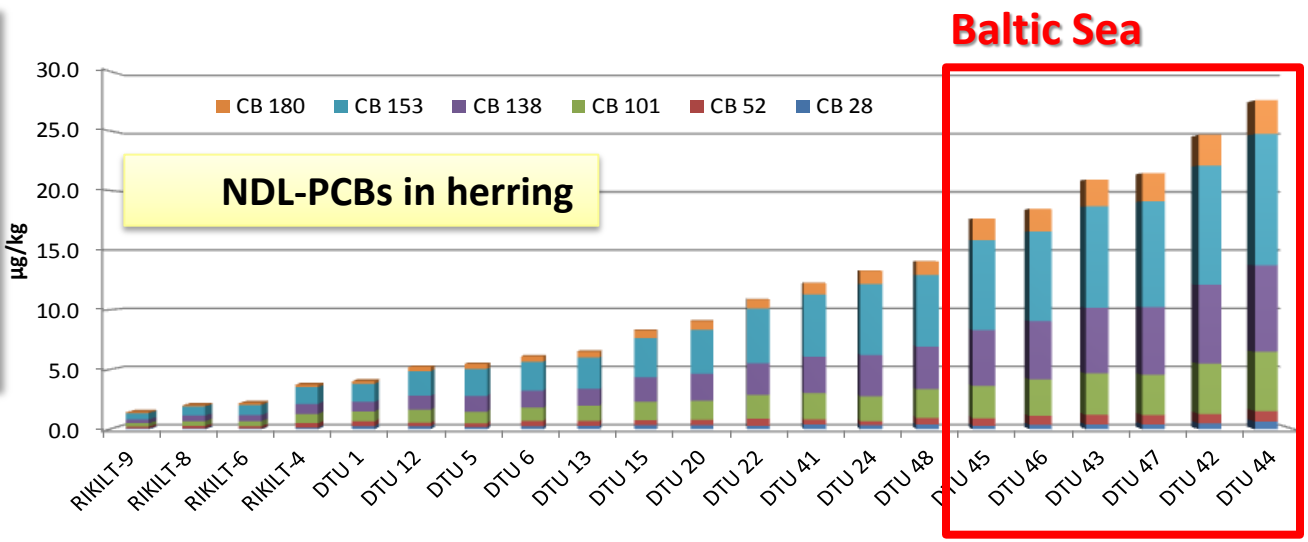
Achievement 4: monitoring survey





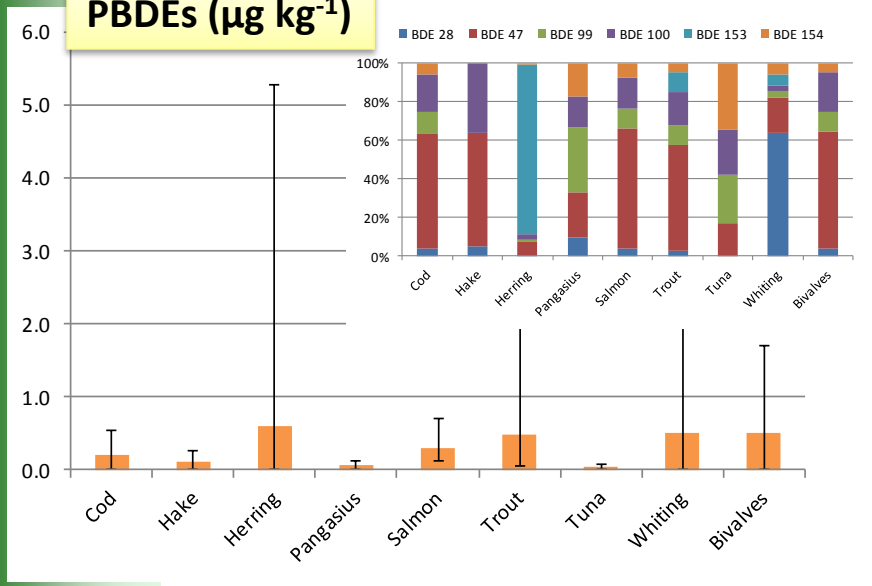
- Occurrence / contamination pattern differs among species / localities / contaminant groups
- Legislation limits not exceeded

Commission Regulation (EU) No 1259/2011 and 853/2011

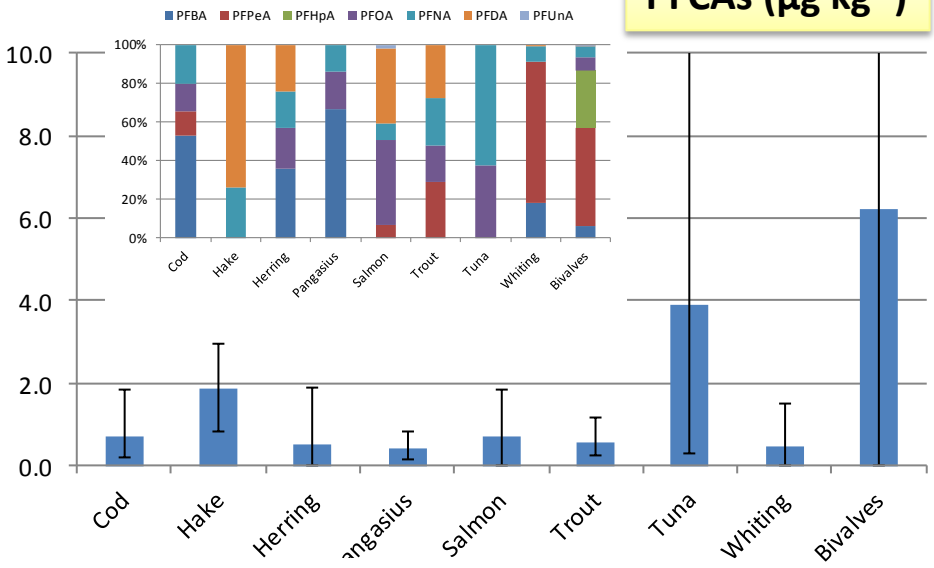


Achievement 4: monitoring survey

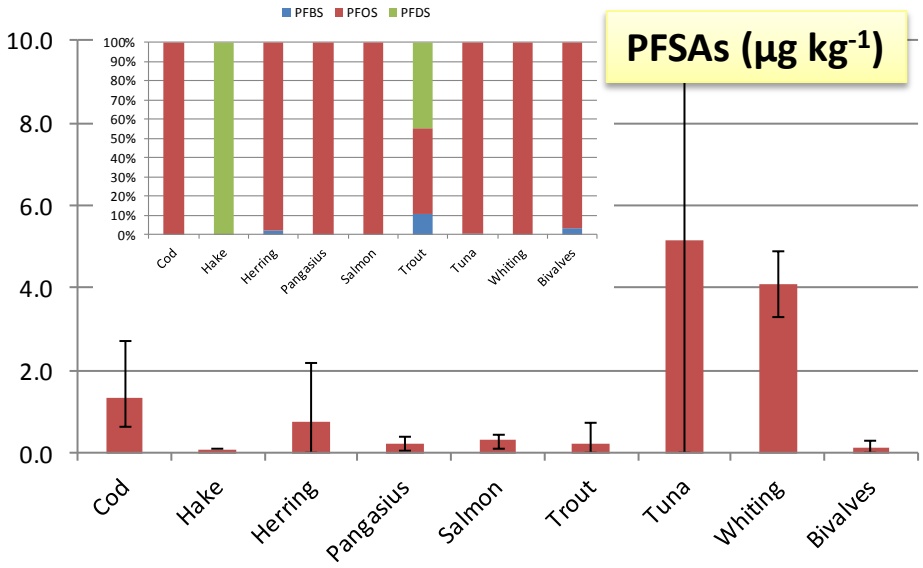
PBDEs (µg kg^{-1})



PFCAs (µg kg^{-1})



PFSAs (µg kg^{-1})



- Occurrence / contamination pattern differs among species / localities / contaminant groups

Achievement 5: DART-MS lipids profiling

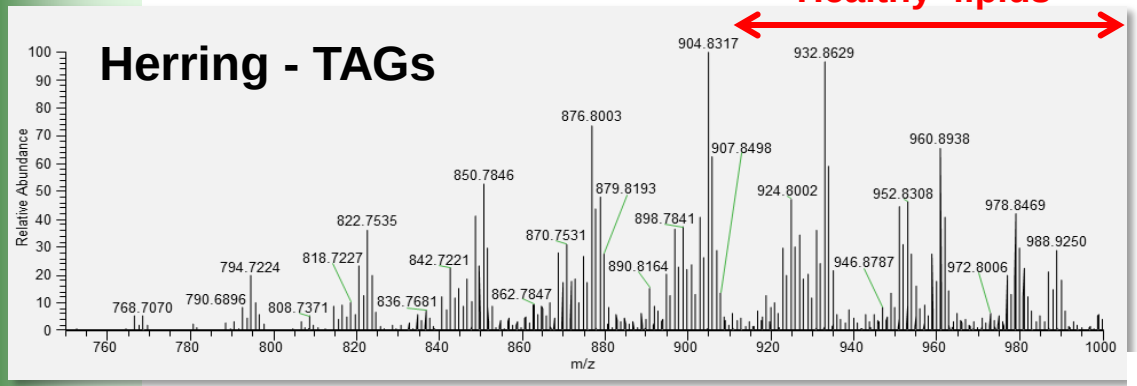
ANALYSIS OF FISH LIPIDS COMPOSITION

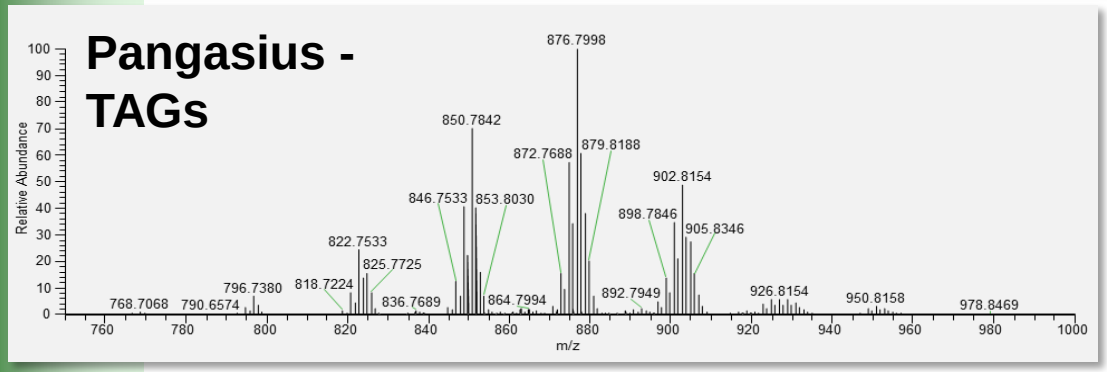
Classic methods: *Isolation of fats* → *Saponification* → *Derivatisation* → *GC-FID*

New method: *Isolation of fats* → *DART-MS*

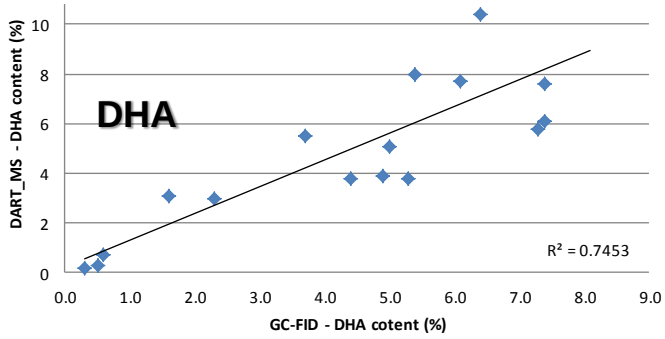
DART(+)-MS profiles

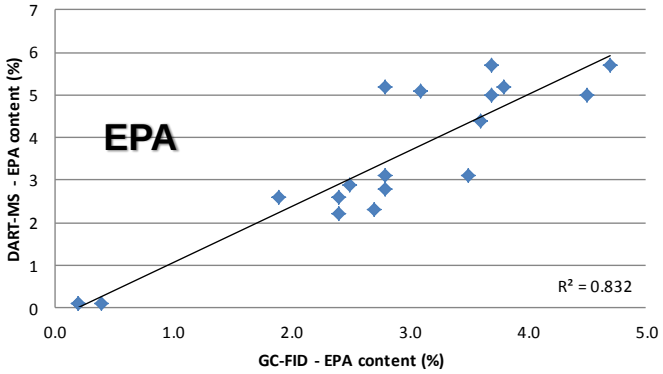
´Healthy´ lipids





Correlation between classic and new method▼





Work package WP1b

Perfluorinated compounds

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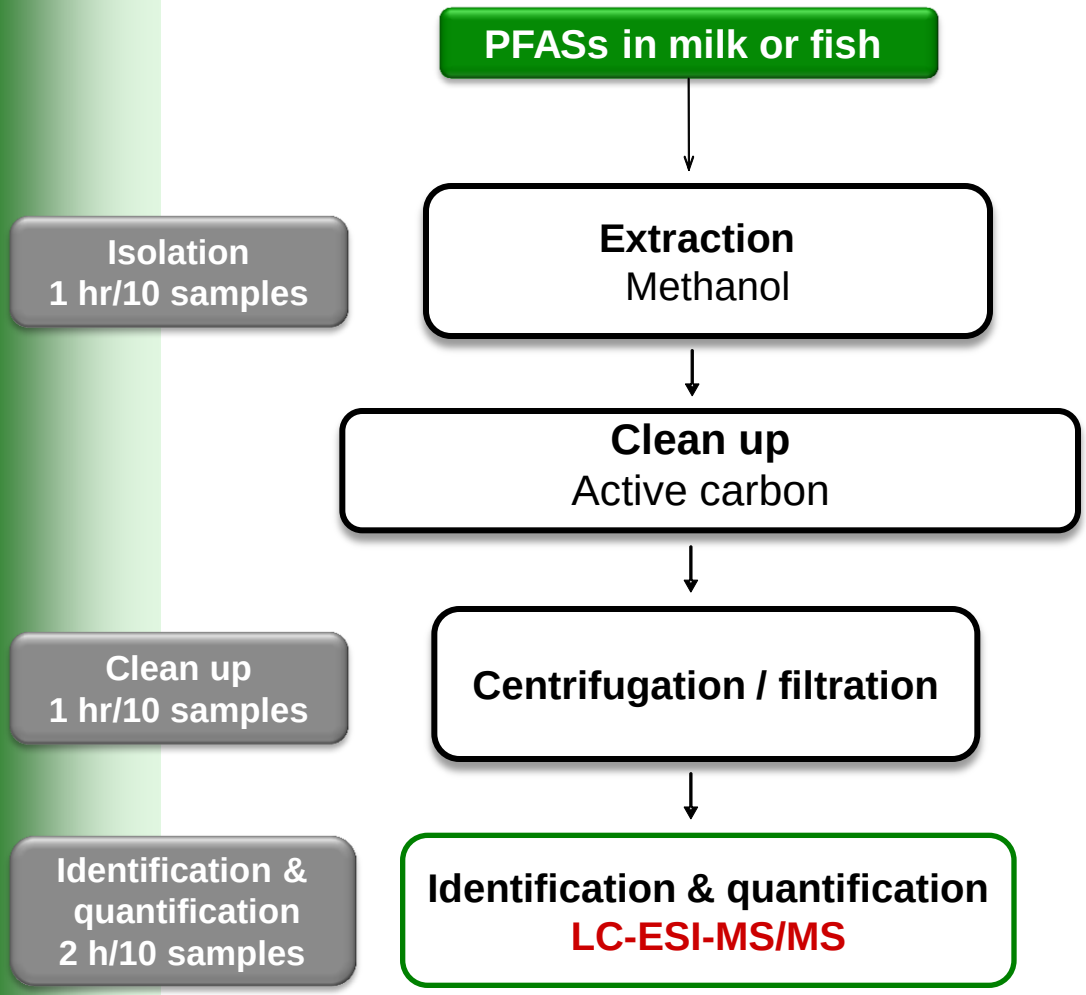
Simplified method for perfluorinated alkyl compounds



TIME SAVING
- Multiple samples at one time

SOLVENT VOLUME REDUCTION

COST/LABOUR SAVING

Sensitivity improved after recent EFSA recommendation

Achievement: full collaborative study

- Simplified method is robust and was transferred to other laboratories (n = 8)
- Works well for most PFASs investigated

Matrix	Analyte	HORRAT	HORRAT evaluation
Fish muscle	PFOS	0.97	< HORRAT ≤ 1.5
	FOSA	0.69	ORRAT ≤ 1.5
Fish feed	PFOS	0.84	0.5 < RAT ≤ 1.5
	FOSA	0.61	0.5 < HO T ≤ 1.5
Milk samples	PFOS	1.42	0.5 < HORR 1.5
	PFOA	0.80	0.5 < HORRAT ≤
	FOSA	2.2	RAT > 2

Work package 1c

development of fast methods for **PESTICIDES** not amenable to multi-residue methods

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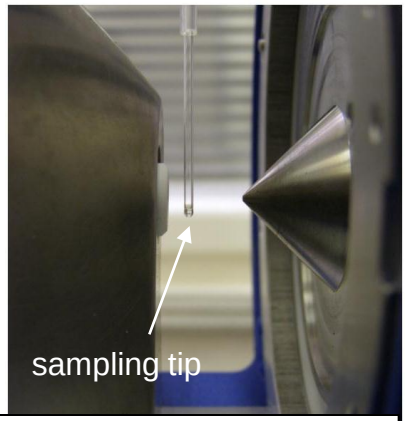
Achievement 1

Dithiocarbamates (DTCs) in fruits/vegetables

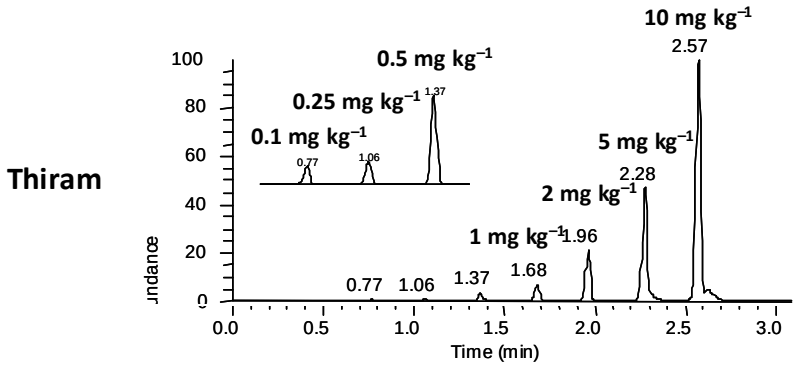
- Issue:
  - Current practice: measured as group after conversion into CS_2
  - EU monitoring: most frequently detected ‘SRM’ pesticide
  - EU-MRL for sum DTCs as CS_2 but separate MRLs for thiram, ziram and propineb [methods laborious or not available]
- CONffIDENCE solution:
  Direct detection by ambient MS (feasibility)



1. surface extraction of fruit with solvent
2. dip glass rod in solvent
3. place rod in front of MS detection device

Achievement 1 *(continued)*

- Straightforward extraction of >10 samples in 15 min
- Detection ~20 seconds/extract



- Sufficiently sensitive and selective at ≤MRL level
- Impact:
  - enables rapid detection/identification of 2 out of 3 targeted DTCs
  - can be used as rapid dedicated method or to supplement existing CS_2-based method to further investigate positive samples

Achievement 2

Paraquat & diquat in potatoes and cereals

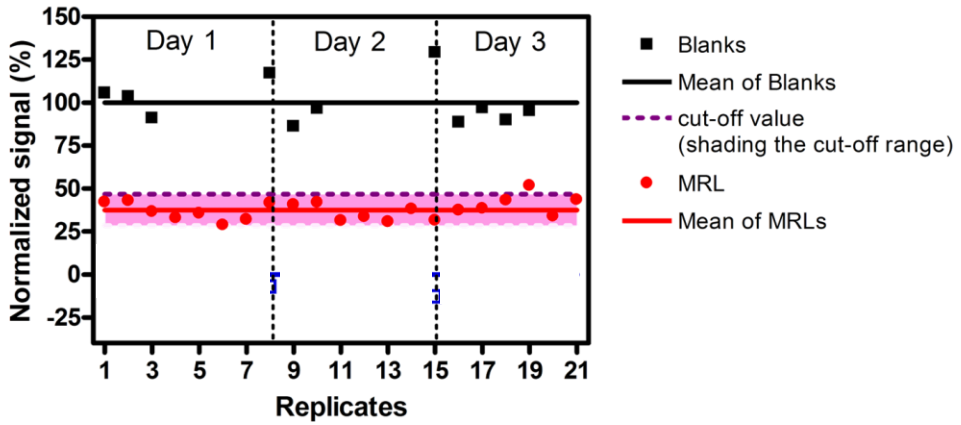
- Issue:
  - widely used as herbicide/desiccant (paraquat now banned in EU)
  - EU-MRL: diquat 0.05*-10 mg/kg, paraquat 0.02* mg/kg
  - extraction/clean up very laborious
  - virtually no monitoring/enforcement data available

- CONffIDENCE solutions:
  - Development of efficient extraction procedure compatible with instrumental and sensor-based detection methods
  - Improved LC-MS/MS method adopted by EURL SRM-pesticides and in use for monitoring
  - Development of immunoassay-based sensor

Achievement 2 *(continued)*

- Electrochemical immunosensor:
  - 2-step process: incubation/washing (~15 min, 10-20 samples parallel); electrochemical measurement 4 min/sample
  - Detection of paraquat, multiplexing possible (demonstrated in multi-class application: detection of mycotoxin DON)
  - Cost of sensor ~3,000 euro

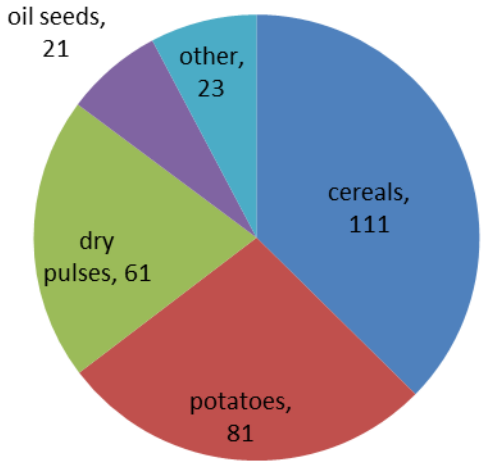
Current data of PQ detection running during 3 days.



- Impact:
  - Novel type of sensor for screening of paraquat, suited for application in basic lab environment
  - Multiplex/multi-class capability demonstrated

Achievement 3: Survey

- Data from survey paraquat & diquat in food and feed:



July 2010-Nov 2012: ~290 samples**			EU-MRL	min	max	median
analyte	product	# positives	mg/kg			
diquat	potato	6	0.05*	0.006	0.021	0.011
	dry pulses (lentils)	6	0.2*	0.021	0.107	0.054
paraquat	not detected	0	0.02*	-	-	-

**Conffidence Survey + additional monitoring by EURL

- Impact: Survey data will be available for EFSA, DG SANCO and EURL Pesticides

Questions ?

Work packages 2a & 2b cluster

Veterinary pharmaceuticals (Coccidiostats & Antibiotics)

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Work package 2a

WP title: Coccidiostats

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WP2a - Coccidiostats

- New relatively fast and inexpensive multiplex method for the screening of:
  - Residues of coccidiostats in eggs

    Commission Regulation (EU) N° 610/2012 amending Regulation (EC) No 124/2009 of 10 February 2009 setting maximum levels for the presence of coccidiostats or histomonostats in food resulting from the unavoidable carry-over of these substances in non-target feed
  - Coccidiostats at cross-contamination levels in non-target feed

    Commission Regulation (EU) N° 574/2011of 16 June 2011 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for nitrite, melamine, Ambrosia spp. and carry-over of certain coccidiostats and histomonostats and consolidating Annexes I and II thereto
- Method developed and validated in-house and through a small-scale collaborative trial
- Prototype developed
- Target analytes: lasalocid A, monensin, salinomycin, narasin, (diclazuril) and nicarbazin
- Target matrices: Laying hens feed and eggs

European Commission

RIKILT INSTITUTE OF FOOD SAFETY WAGENINGEN UR

CER Groupe

fera The Food and Environment Research Agency

Queen's University Belfast

nutreco

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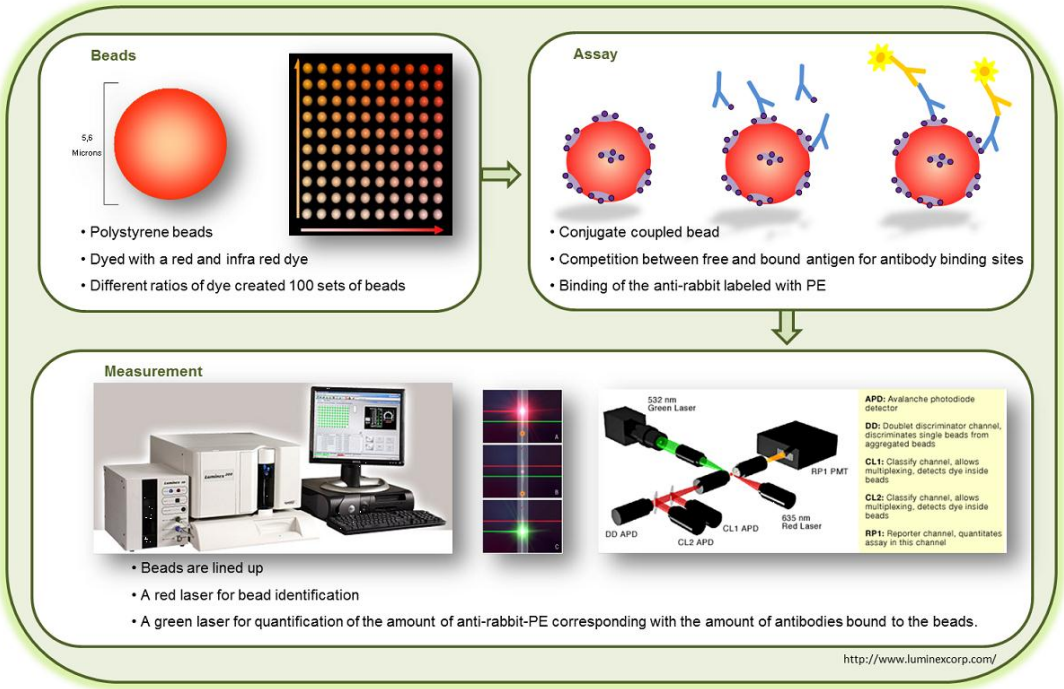
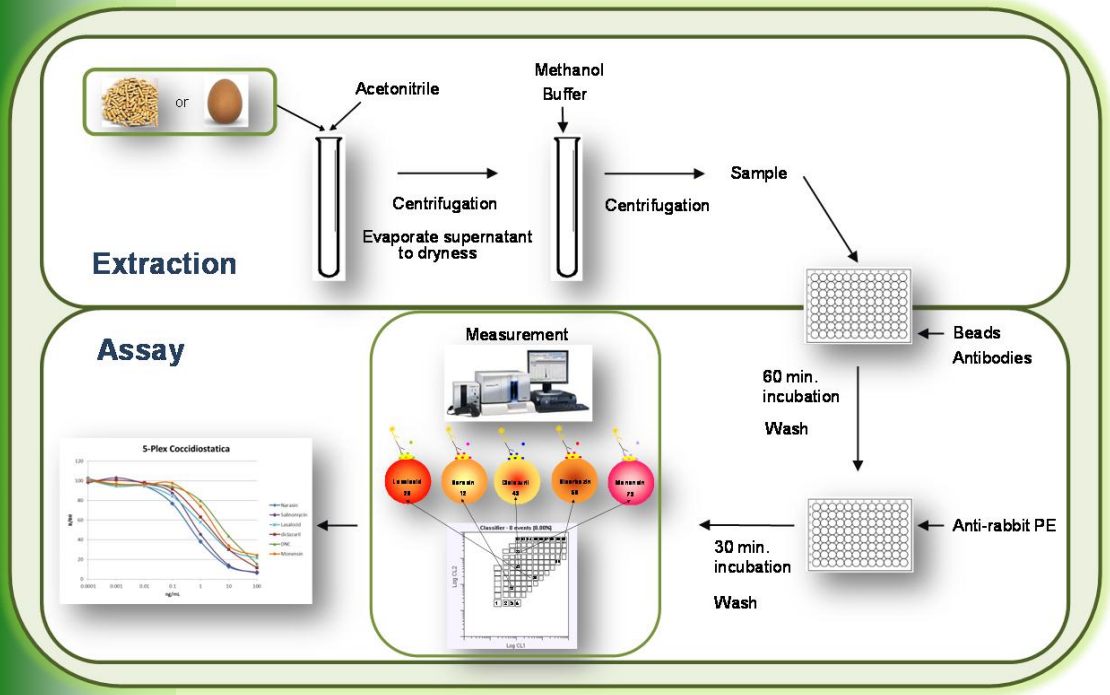
The Technology

Flow cytometry based multiplex immunoassay





The Method

Generic extraction
40 samples (240 analytes) per day in routine

Ring trial

- 9 egg materials
- 16 feed materials
- Analysis in blind quadruplicate;
- each replicate analysed in duplicate
- 5 participating laboratories

Ring trial - Overview

	Eggs	Feed
Narasin/Salinomycin	2.89	0.52
Lasalocid	0.17	2.75
DNC	0.35	10.47
Diclazuril	9.14	93.40
Monensin	2.42	1.65

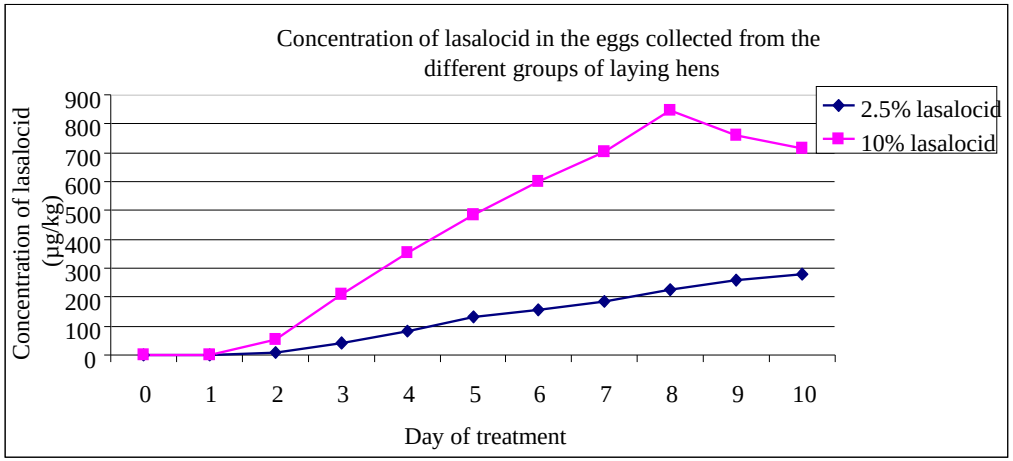
Rate of false positives in the blank in %

Established at 95% confidence level (maximum rate of false negatives is 5%)

WP2a – Carry-over study

- Lasalocid: from laying hens feed to eggs
- Four groups – 30 to 55 weeks:
  - Control,
  - 10% of the authorised additive level,
  - 2.5% of the authorised additive level
- Samples tested by:
  - FCIA and
  - LC-MS/MS: reference method

$\Rightarrow$ results in accordance



WP2a – Carry-over study - Conclusions

- Linear relationship between lasalocid contaminated feed and lasalocid residues in eggs verified

$$\text{concentration in eggs } (\mu g\, kg^{-1}) = 63.6 \times \text{concentration in feed } (mg\, kg^{-1})$$
$$r = 0.999$$

- Rapid and reliable estimation with FCIA (as for LC-MS/MS)

Work package 2b

WP title: Antibiotics

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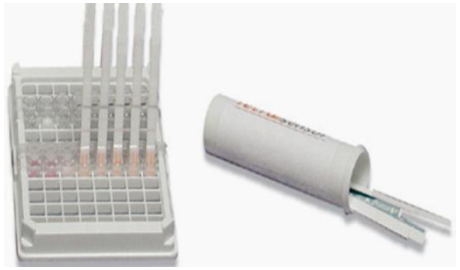
WP2b - Antibiotics - achievements

- Development, validation and impact demonstration of dipsticks (DS) to detect **antibiotics** in a range of matrices

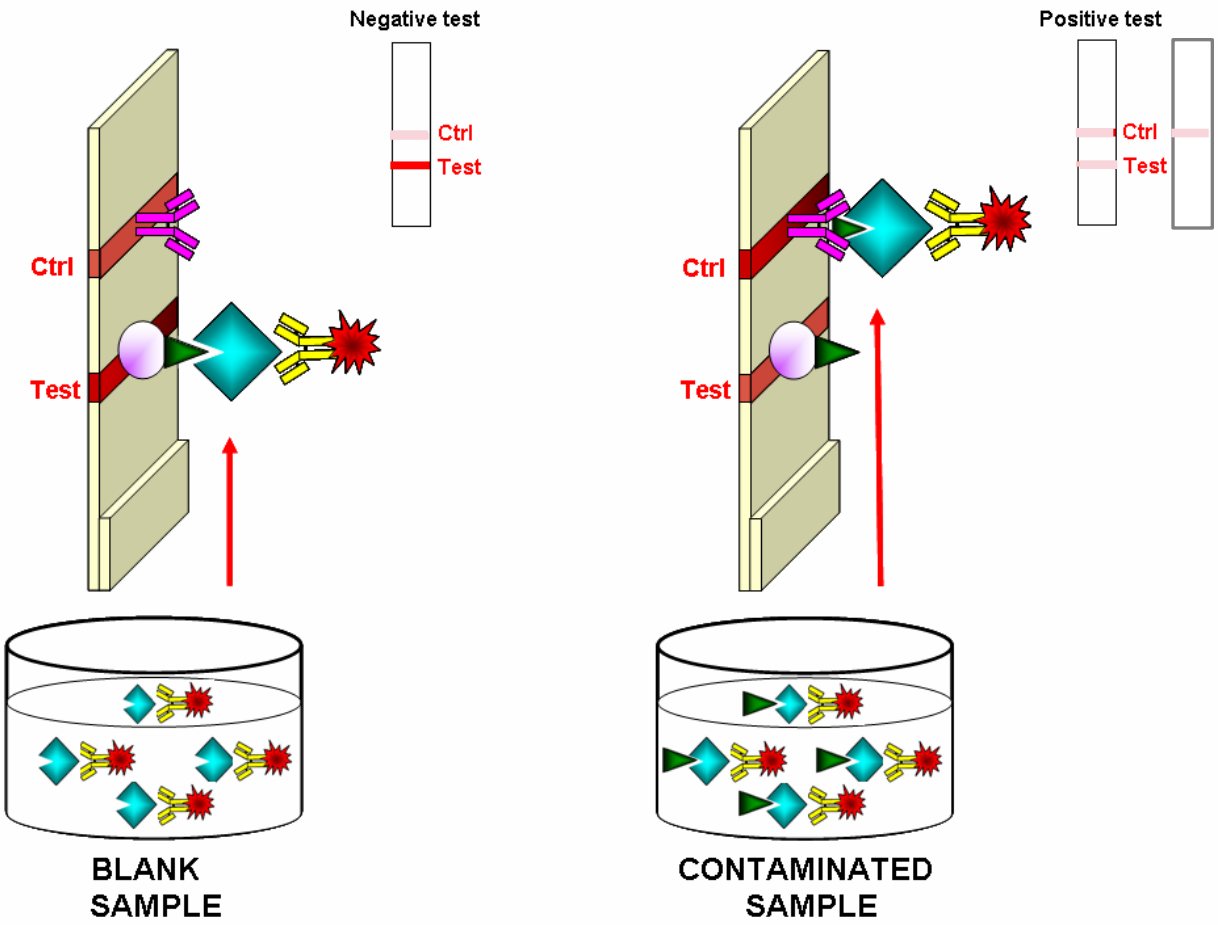


- Multiplex DS
  - Sulfonamides, fluoroquinolones, tylosin and chloramphenicol in honey

- Single-component DS
  - Tetracyclines (TCs) in feeds, urine and processed meat

CER Groupe
Nestlé Research™
fera The Food and Environment Research Agency
CONFIDENCE
unisensor
CSIC Consejo Superior de Investigaciones Científicas

Dipstick principle



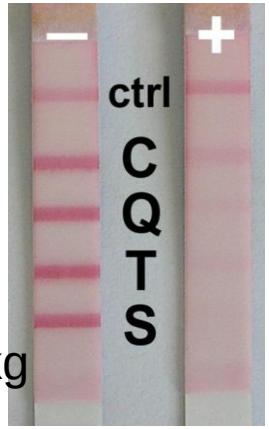
Multisensor – bee4sensor for honey

- **Multisensor**: Unique **multiplex,** antibody based lateral flow dipstick **assay**, for the screening of **sulphonamides**, **fluoroquinolones**, **tylosin-A**, and **chloramphenicol** in **honey**

- Laboratory method
  - Single laboratory validation
  - Inter-lab validation with 7 European laboratories
- Field-test method
  - Proof of principle
  - Global field trial with 16 participants from across governmental, industrial organisations and academia
- Rapid test for industry and enforcement authorities

Comparison of detection capability

Antimicrobial	LC-MS/MS [µg kg^{-1}]	Multi-lab [µg kg^{-1}]	Multi-field [µg kg^{-1}]
Sulfathiazole	2.5	25*	50
Tylosin	2	10	25
Ciprofloxacin	10	25#	25
Chloramphenicol	0.15	5	100
Time required	**3x2 Days**	**Less than 4h**	**less than 1h**



*Applicable to 11 other sulfonamides
#Applicable to 5 other fluoroquinolones and 3 at 100 µg/kg

Multisensor – bee4sensor for honey

The test kit (bee4sensor) is already marketed by unisensor and will be soon produced, based on customer demand: http://www.unisensor.be/en/catalog/antibiotics-28/bee4sensor-45.php

Tetrasensor for feed, urine and meat

- **Tetrasensor**: Extension of the existing concept of a receptor based lateral flow dipstick assay for **tetracyclines** in milk, honey, raw animal tissues to **feed**, **urine** and (**cooked) meat**

- Following the assay optimisation a single laboratory validation (CD 2002/657/EC) was performed

- This rapid, sensitive and easy to use test is capable of the detection of tetracycline compounds in a range of matrices at the required detection limit of 100 µg kg^{-1} and lower

- 30 to 50 samples can be analysed in a day (4 to 7 samples per h)

Conclusions and Summary

Coccidiostats in egg and feed:

- A new multiplex method has been validated via a inter-laboratory ring trial. Good data obtained with the exception of a high false positive rate for diclazuril in feed.
- Relationship between lasalocid contaminated feed and lasalocid residues in eggs determined

Antibiotics in honey:

- Successful development and validation of a rapid method *for use in the laboratory or the field.* Commercialisation underway.

Tetracyclines in meat and urine:

- Successful development and validation of a rapid method at the required detection limit of 100 µg kg^{-1}

Questions ?

Work package 3

Heavy metals

CONffIDENCE Final Stakeholder Workshop
Brussels, 18 December 2012

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WP3 – setting the scene

- Current situation in EU legislation:

Foodstuffs
MLs for Pb, Cd, Hg and Sn
EU directive 2006/1881/EC (and amendments)

Animal feedingstuffs
MLs for As, Pb, Cd and Hg
EU directive 2002/32/EC (and amendments)

Only maximum levels for total concentration of the metals

CONffIDENCE - progress beyond state-of-the-art

Arsenic

- inorganic As (iAs) is the toxic form of As
- Lack of specific data on iAs (*EFSA, 2009 and JECFA, 2011*)
- Lack of validated, standardised methods *(EFSA, JECFA)*

Focus on inorganic arsenic

Mercury

- Methylmercury is more toxic than inorganic Hg
- Call for methods for specific MeHg determination *(EFSA)*

Focus on methylmercury

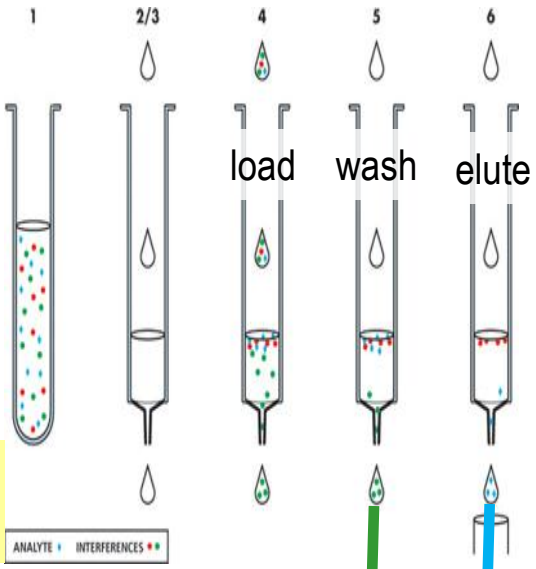
Seafood/marine feed

- Seafood is the predominant source of As and Hg in the European diet
- Focus on marine feed and food sample types

SPE-HG-AAS – a novel speciation alternative…

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

Inexpensive detection system



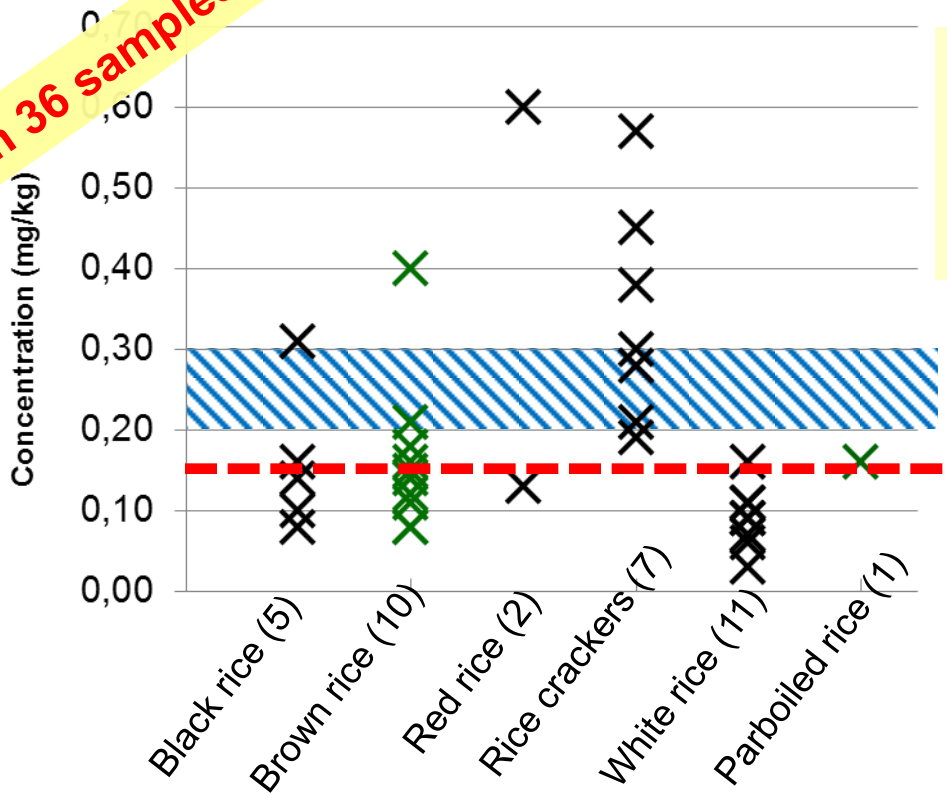
Concept: Sequential elution for selective separation of **inorg As** from **organoAs** species by SPE

- Survey on inorganic arsenic in seafood (N=148)
  - Fish samples (N=121) <0.01 - 0.04 mg/kg
  - Bivalves (N=27) <0.01 - 0.07 mg/kg
- Input to EFSA evaluation on inorganic arsenic exposure
- Important info for seafood producers and authorities

Inorganic arsenic in rice – a hot food safety topic

- Tailoring of SPE HG-AAS method for rice and rice products
- Simplified extraction in waterbath for increased sample throughput (> 50 samples extracted in 1 hour)

Survey on 36 samples



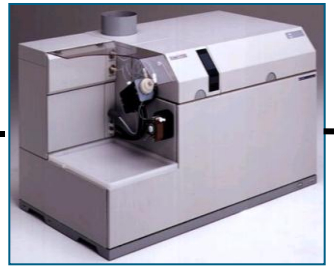
Dataset provides input to:
EFSA (exposure estimation)
EU commission (legislation?)
CODEX (legislation?)

Future EU max level (0.2-0.3 mg/kg)?

Chinese max level (0.15 mg/kg)

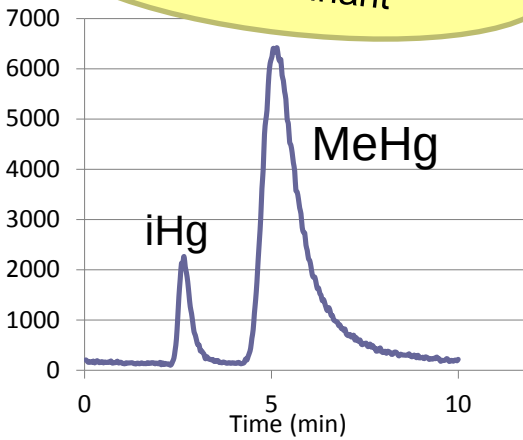
Speciation analysis of mercury by HPLC-ICPMS

High capacity!



Ultra-sonification **HPLC** **Column** **ICPMS**

MeHg is a priority contaminant

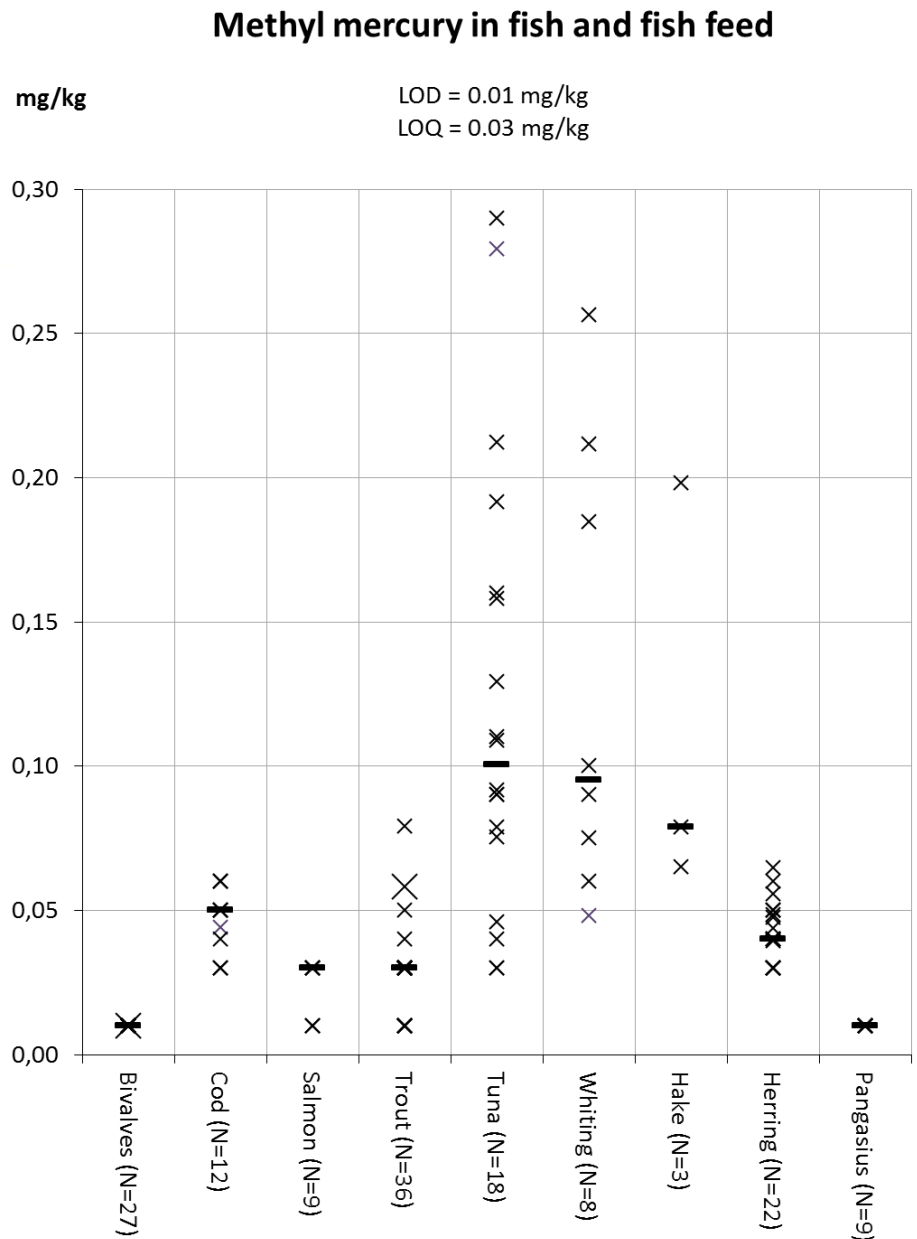


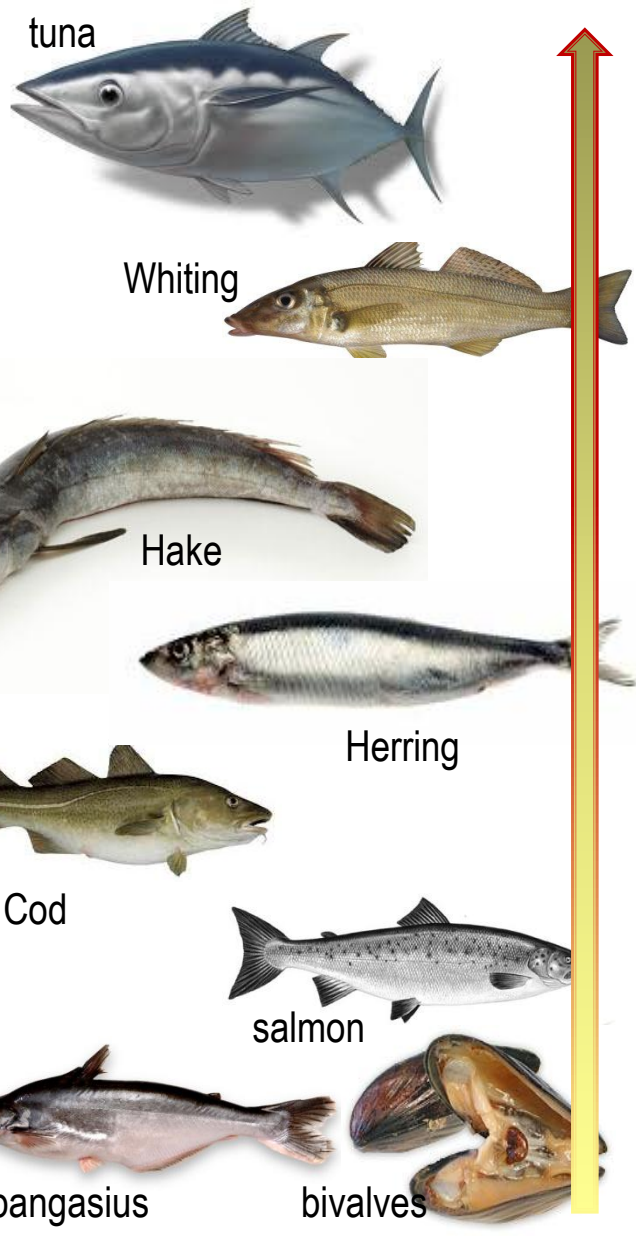
Chromatogram of fish muscle sample

- Simple extraction by ultrasonification for increased sample throughput (> 50 samples extracted in approx 1 h)
- Validated for marine food and feed

- Applied on a range of seafood and marine feed samples
- Dataset is a valuable input for ongoing risk assessment on MeHg
- Method useful for both food/feed control and industry

Survey data – MeHg in seafood





Output from CONffIDENCE WP3

Methods:

- iAs in marine samples by SPE HG-AAS
- iAs in rice samples by SPE HG-AAS
- MeHg in marine samples by HPLC-ICPMS

Candidate methods for future food and feed control purposes

Alternative speciation approach
Simple, high-throughput extraction procedures

Collaborative trials:

- iAs in marine samples by SPE HG-AAS (10 labs)
- MeHg in marine samples by HPLC-ICPMS (4 labs)

Survey data:

- iAs in marine samples (N=148)
- iAs in rice samples (N=36)
- MeHg in marine samples (N=148)
- MeHg in marine feed (N=26)

Dataset input to ongoing exposure assessments

Contribution to risk-benefit analysis :

- Seafood samples analysed for contaminants and
- Reported to EFSA databases for future risk evaluation

Dataset input to ongoing risk-benefit assessments on marine food/feed

Questions ?

Work package 4a & 4b & 4c cluster

Biotoxins: alkaloids, marine biotoxins, mycotoxins

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Work package 4a

WP title: Alkaloids

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Brussels, 18 December 2012




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Achievement 1: Dipsticks

- Tropane alkaloids (TAs) in feed and ergot alkaloids (EAs) in cereals and feed (Unisensor)
- Fast extraction; low price (ca. 5-7 €); fast result (30 min)
- Over 60 samples / day / analyst

4 g of test portion

+ 40 ml MeOH/H_2O/HCOOH (60:40:0.4)

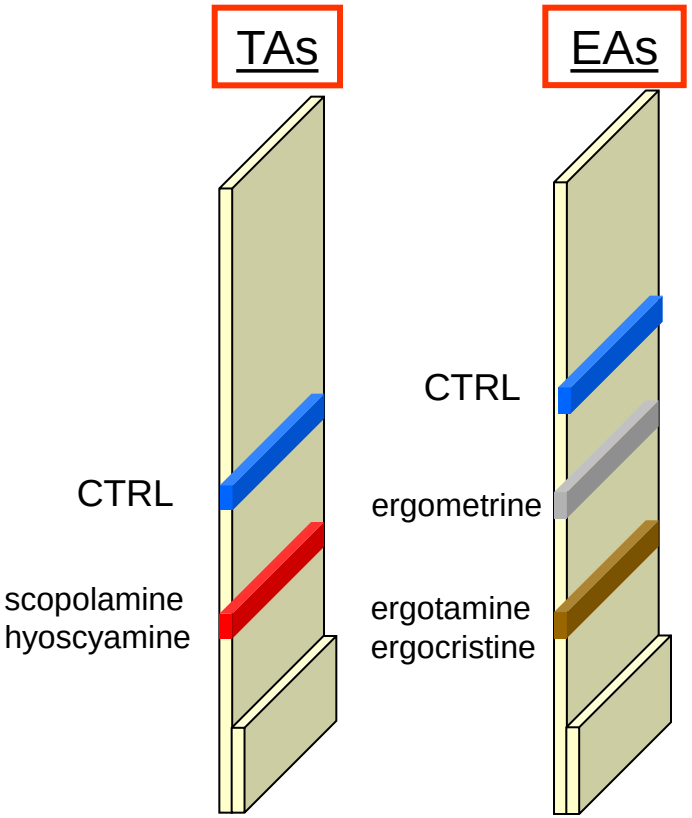
Vortex 2 min

Dilute 5 times in buffer (EAs)

or

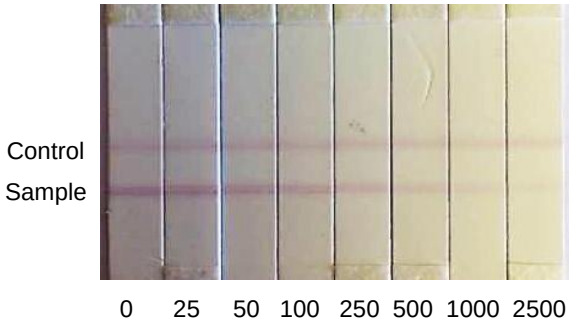
Dilute 10 times in buffer (TAs)

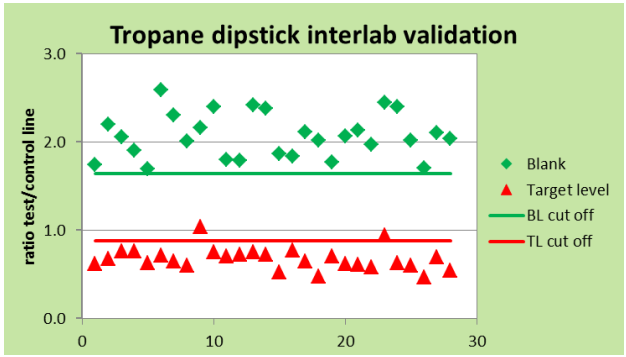
Run the dipstick test



Example: Tropane alkaloids dipstick

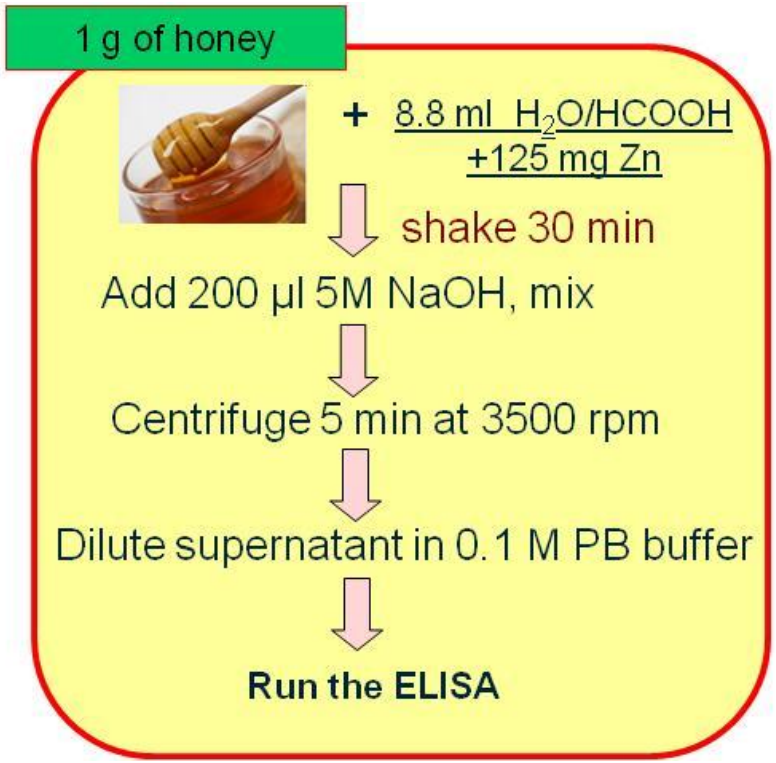
- In-house validated (RIKILT)
  - Target level: 800 µg/kg hyoscyamine / scopolamine
  - 21 Blank feed samples
- Inter-laboratory validated
  - 7 Labs participating
  - Successful results
- Application
  - Design allows field testing (e.g. HACCP plan Nutreco)
  - Screening: over 95% of feed samples are blank

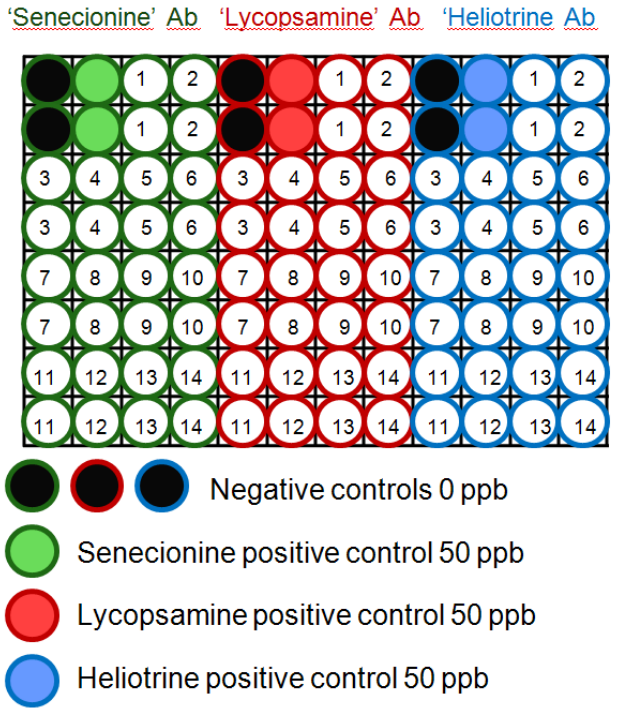




Achievement 2: Multiplex ELISA

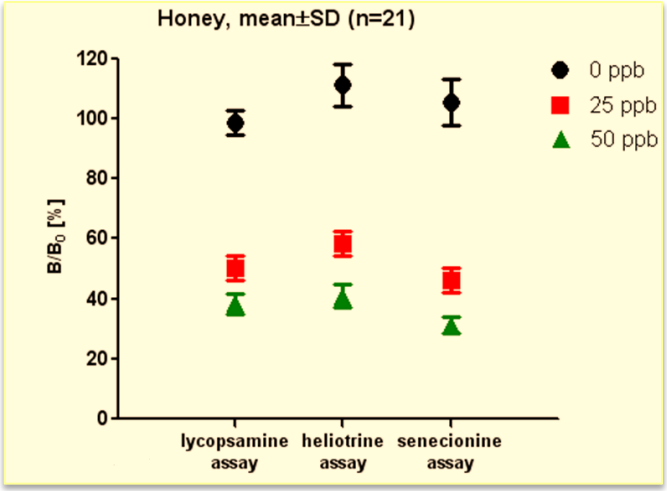
- Pyrrolizidine alkaloids (PAs) occur in e.g. weeds, herbs, honey
- ELISA screening of major PAs in honey and feed (QUB)
  - In parallel screening for lycopsamine, heliotrine and senecionine
  - Cross reactivities to structural analogues
  - Results in 2 h; over 50 samples / day / analyst





Example: PAs in honey

- In-house validated (QUB)
  - Target level: 50 µg/kg senecionine, lycopsamine, heliotrine
  - 4 Blank honeys
- Method verification
  - 3 laboratories, on-going
- Applications
  - On (production) site testing of raw honeys
  - Ca. 10% of honeys will test positive



Achievement 3: NIR method

- NIR hyperspectral imaging method to detect and quantify ergot bodies in cereals at levels below regulatory limits (CRA-W)
- Full conveyer belt system with belt speed of 100 mm/s allows analysis of up to 100 kg grain/hour

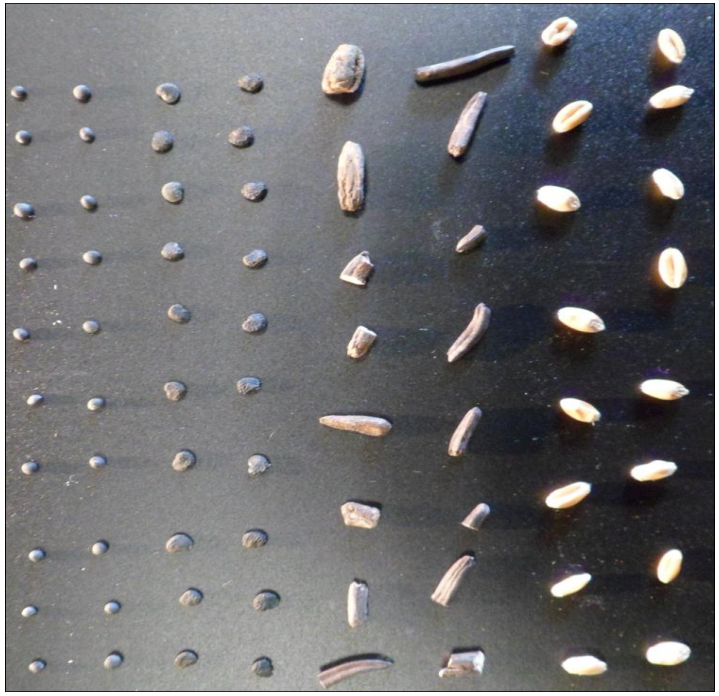
NIR line scan imaging system

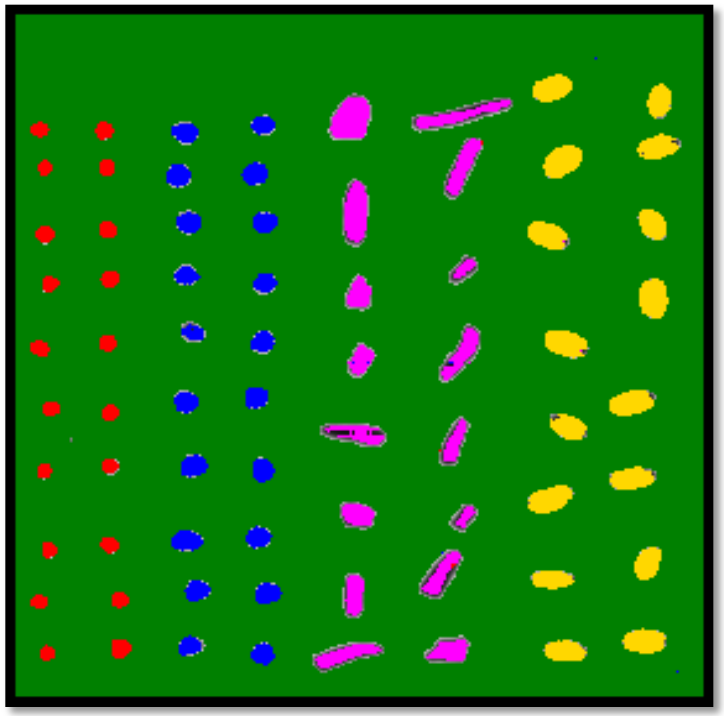
Conveyor belt

Test system in operation at Nutreco (2011)

Further developments

- Multicontaminants detection: ergot, Datura, …





2 lines of rapeseed, Datura seeds, ergot sclerotia and wheat kernels, respectively

Work package 4b

WP title: Marine Biotoxins

CONffIDENCE Final Stakeholder Workshop
Brussels, 18 December 2012



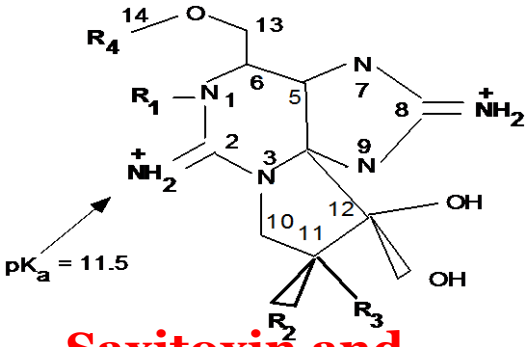

SEVENTH FRAMEWORK PROGRAMME

Focus

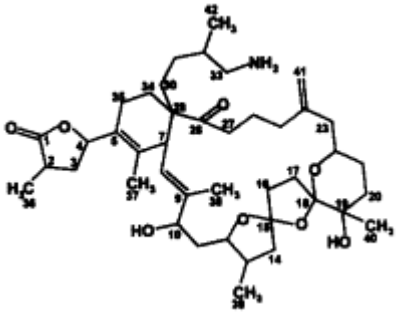
Focus of the research has been on a range of regulated and emerging marine biotoxins

These are naturally occurring chemicals produced by algae that accumulate in shellfish

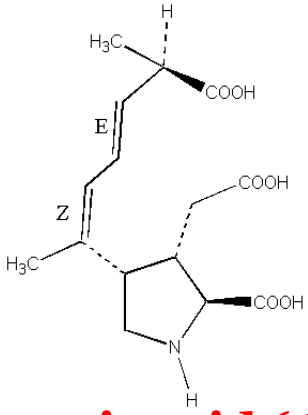
The Marine Toxin Targets



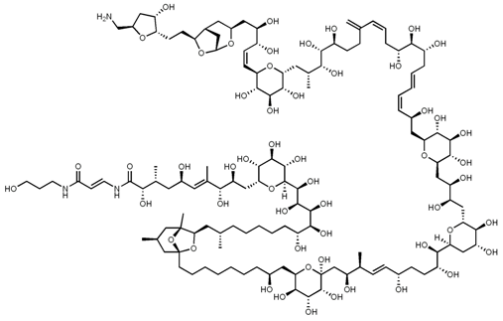
Saxitoxin and analogues (PSP)



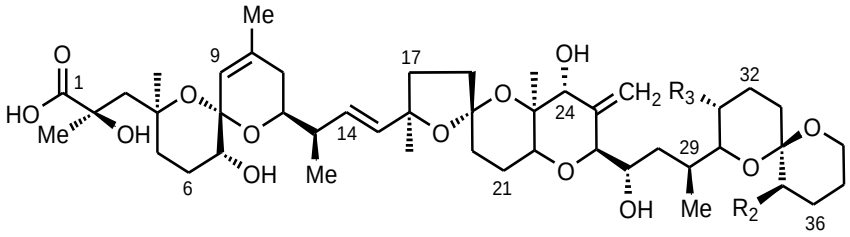
Spirolides



Domoic acid (ASP)



Palytoxin



Okadaic acid and analogues (DSP)

Major Achievements

Conffidence research (and researchers) contributes to two EFSA opinions on emerging marine toxins

EFSA Journal 2009; 7(12):1393

SCIENTIFIC OPINION

Scientific Opinion on marine biotoxins in shellfish – Palytoxin group[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

EFSA Journal 2010; 8(6):1628

SCIENTIFIC OPINION

Scientific Opinion on marine biotoxins in shellfish – Cyclic imines (spirolides, gymnodimines, pinnatoxins and pteriatoxins)[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

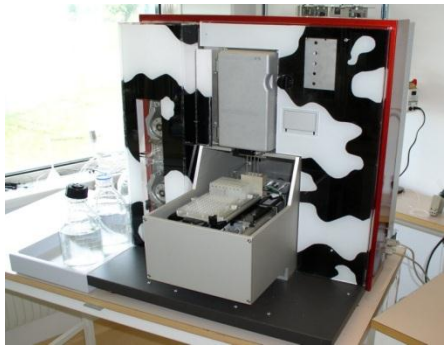
This scientific opinion replaces the earlier version published on 7 June 2010.[4]

Major Achievements

The monitoring for marine biotoxins is a global issue.

Performing fast, low cost testing not requiring animals is an important issue

World's first multiplex biosensor assays for multiple shellfish toxins developed and validated



ELSEVIER

Biosensors and Bioelectronics

Volume 26, Issue 6, 15 February 2011, Pages 3029–3036

Use of a novel micro-fluidic device to create arrays for multiplex analysis of large and small molecular weight compounds by surface plasmon resonance

Hot of the press

World's first low cost, commercially available, multi-toxin detection assay under development (*planer waveguide based detection system on a customised biosensor device*)

New biosensor SME to be established to exploit Conffidence marine toxin research

Chip based technologies will be central to a new generation of food safety testing

Work package 4c

WP title: Mycotoxins

CONffIDENCE Final Stakeholder Workshop
Brussels, 18 December 2012




SEVENTH FRAMEWORK PROGRAMME

WP4c: mycotoxins

Commodity dedicated **multiplex dipstick tests** for the determination of major ***Fusarium*** **toxins**

WHEAT

OAT

WHEAT BASED BREAKFAST CEREALS

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

MAIZE

MAIZE FEED

MAIZE BASED BREAKFAST CEREALS

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

Target levels: EU maximum permitted levels

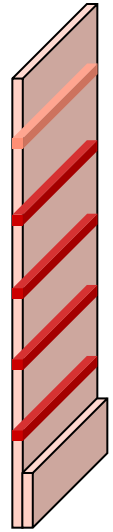
The assay procedure

Methanol/water extraction

Dilution with buffer

Incubation at 40°C, 10 min
Migration, 10 min

Reading



NEG: Test Lines **darker** than CTRL line

POS: test Lines **lighter** than CTRL line

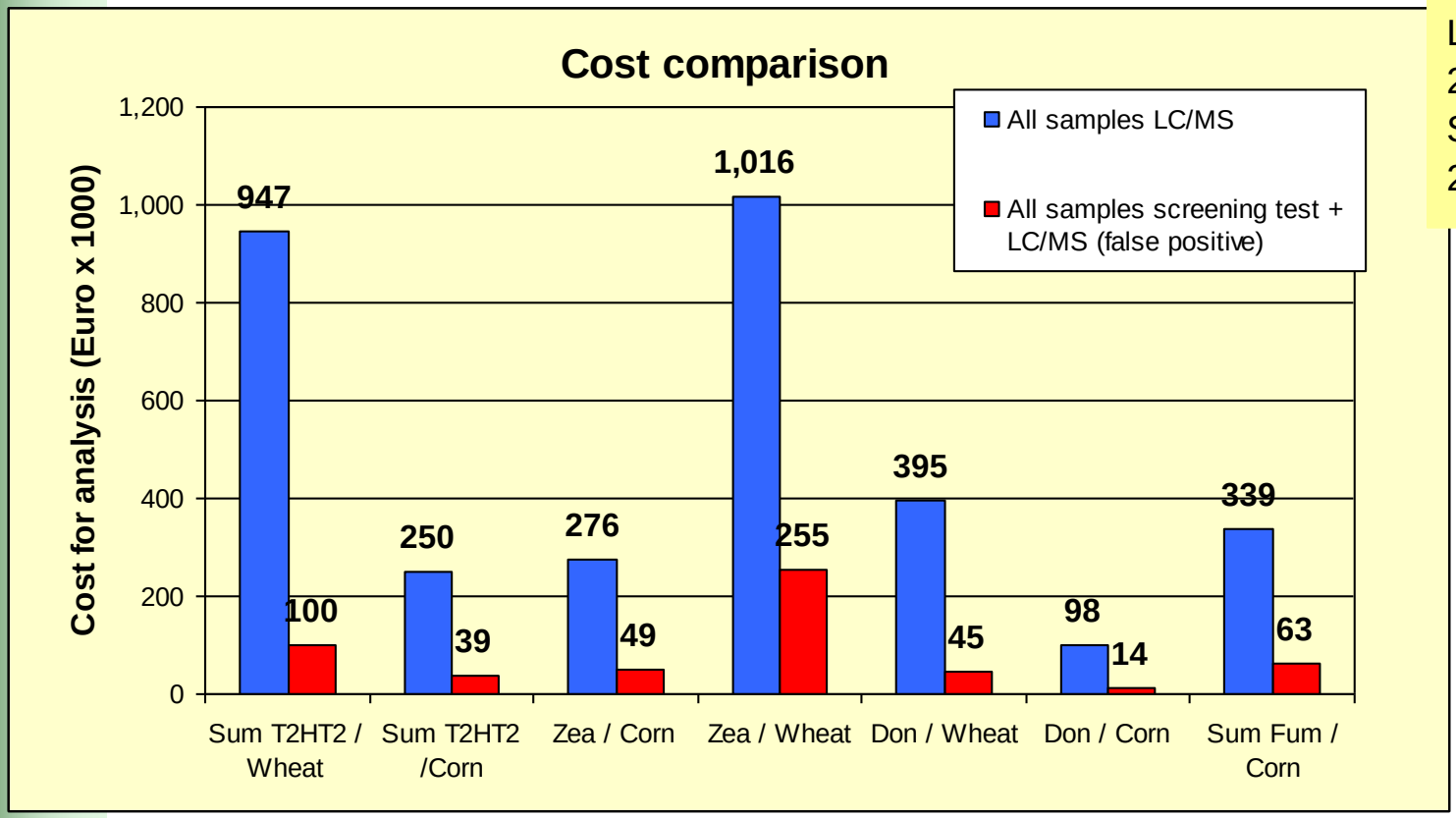
Total analysis time: 30 min for 6 mycotoxins

In house validation: main results

- Evaluation of error contributions from various factors (day, matrices….) proved the **ruggedness of the assay.**
- The test can differentiate blank samples from samples contaminated at target levels with **false positive rates less than 6%.**
- When tested with a set of naturally contaminated samples the assay showed **good agreement with LC-MS/MS** confirmatory method and **NO false negative** results.

We checked fitness for purpose by considering the cost situation **and** expected frequency distribution of target analytes

Estimating total analytical costs for two options:
- Analysing all samples with LC/MS
- Analysing all samples with the screening test and all false positive additionally with LC/MS



LC/MS:
200 Euro/sample
Screening test:
20 Euro/sample

The test presented here is considered fit for purpose

The commercial kit

unisensor

www.unisensor.be

MULTIPLEX: **6** mycotoxin analysed in **1** test

FAST: up to **8 samples** in **1 hour** (including sample preparation)

SENSITIVE: mycotoxin detection at levels close to EU regulatory limits

USER FRIENDLY: **5 min** for sample preparation, easily performed on site

Impact

- Quality control of incoming raw materials in food companies and feed mills

- Quality control of raw materials at the farm (taking into account that the importance of rapid test will shift from feed mill towards farm)

In progress….

Satisfactory results from in house validation

Kit commercially available (standardized large scale production)

Follow up:

Full-scale interlaboratory validation

- **Number of participants:** 13 Laboratories
- **Matrix/mycotoxin combinations:**

Wheat: DON, ZEA, T-2, HT-2

Maize: DON, ZEA, T-2, HT-2, FB_1, FB_2

- **Expected outputs:**

Precision profile under reproducibility conditions

Incidence of false positives

small scale
interlaboratory validation requested by the contract

results expected by December 31, 2012

Questions ?

Work package 5

Dissemination and exploitation

CONffIDENCE Final Stakeholder Workshop
Brussels, 18 December 2012

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Main outputs

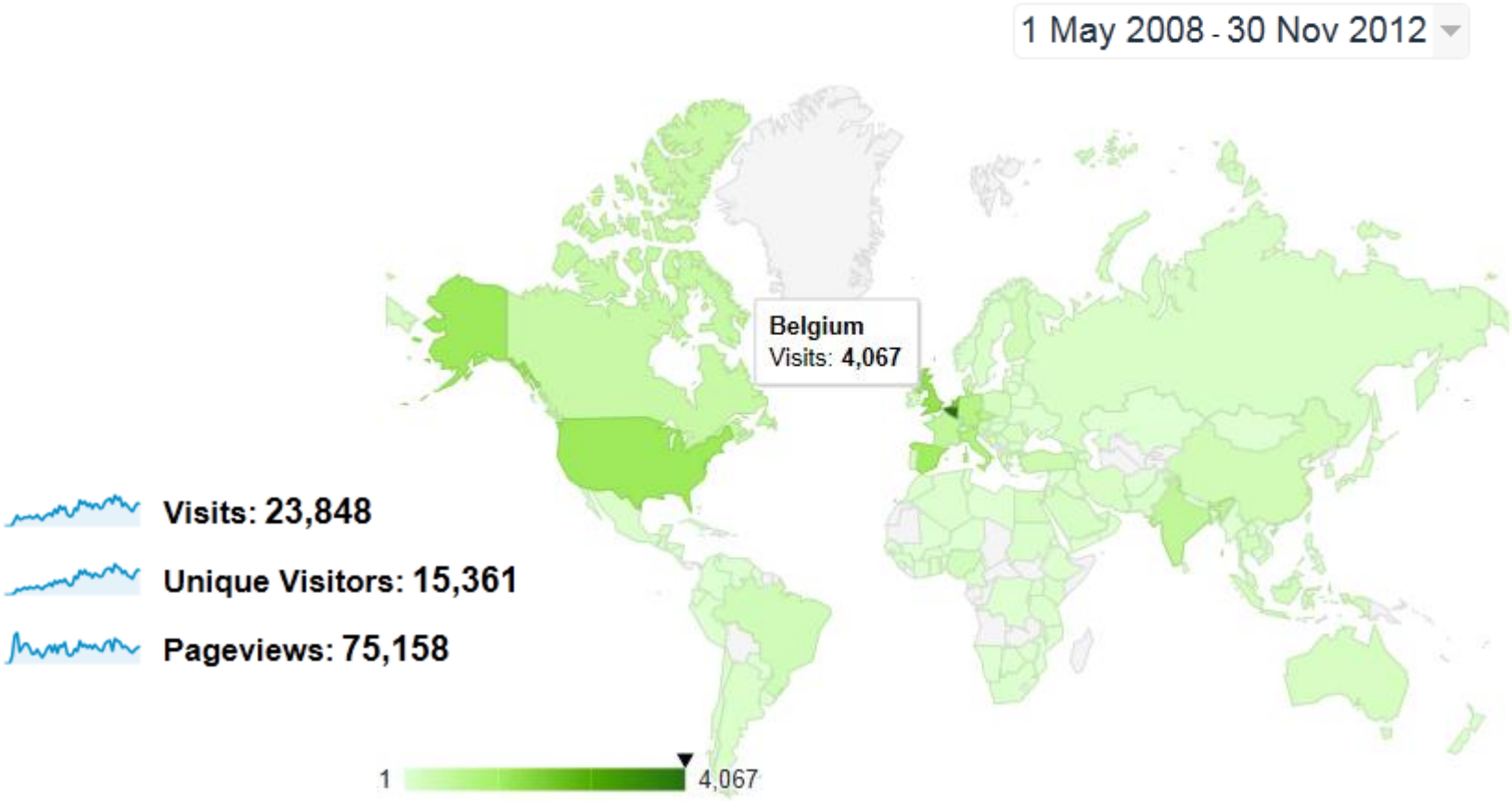
- Website, e-Newsletters
- Open days, workshops, courses
- Lectures, posters, publications

Public website

- 111 events
- 48 news

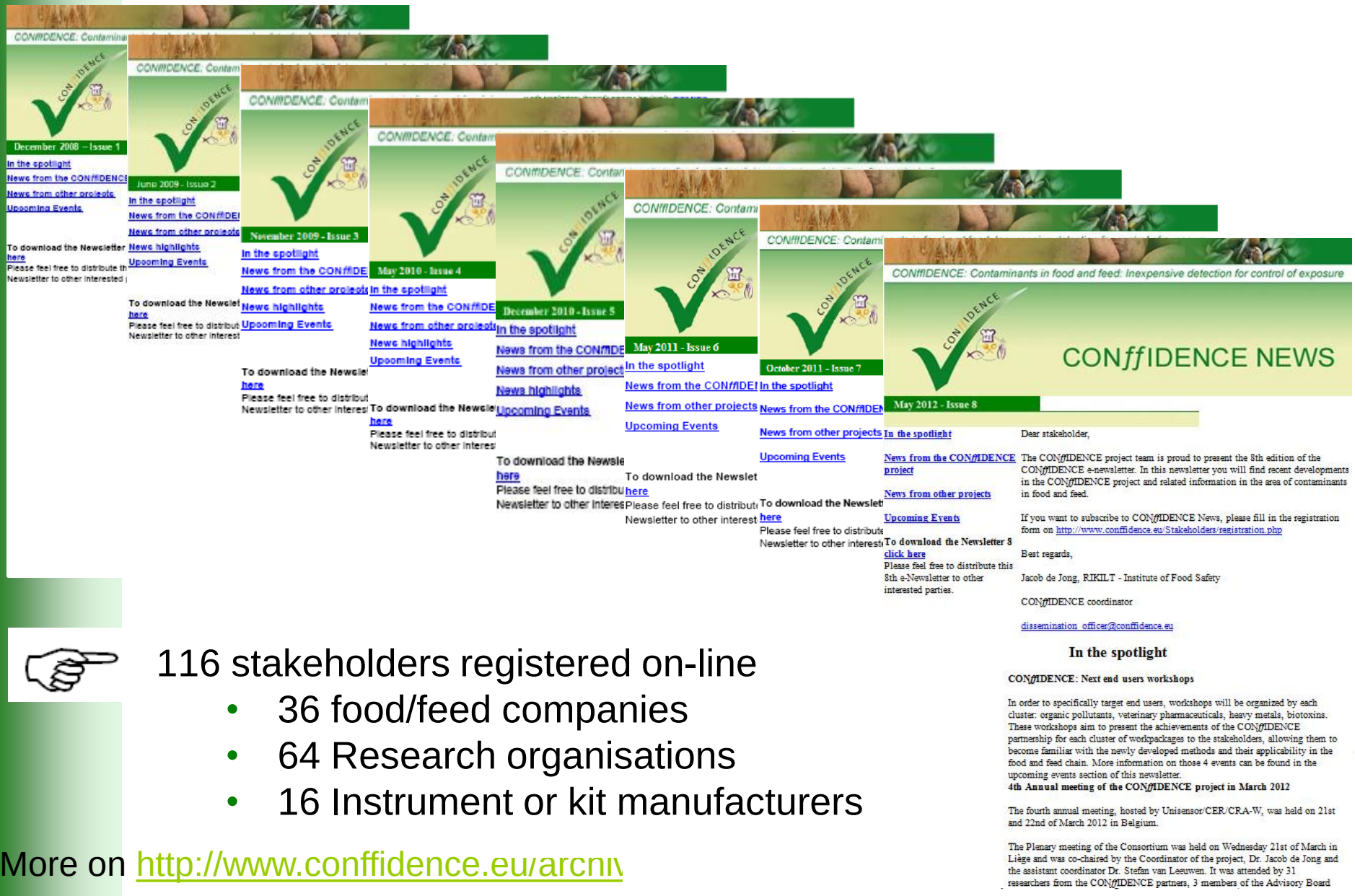
More on http://www.conffidence.eu/

Public website



- 1370 pages viewed by month
- 430 visits by month
- 10% by CONffIDENCE partners

8 e-Newsletters



116 stakeholders registered on-line

- 36 food/feed companies
- 64 Research organisations
- 16 Instrument or kit manufacturers

More on http://www.conffidence.eu/arcniv

3 Open Days

- 1st Open Day: RME

Noordwijkerhout (NL), 27 January 2010

- 2nd Open Day: World aquaculture

Natal (Brazil), 9 June 2011

- 3rd Open Day: RAFA

Prague (CZ), 3 November 2011

260 Participants

14 Oral presentations, 55 posters, 12 demonstrations

More on http://www.conffidence.eu/Project_output/openday.php

4 Stakeholders workshops

- Cluster 1: LC/MS/MS workshop
  Barcelona (SP), 3 July 2012

- Cluster 2: EURO Residue VII
  Egmond aan Zee (NL), 16 May 2012

- Cluster 3: EURL Heavy metals
  Brussels (BE), 20 Sept 2012

- Cluster 4: WMFmeets IUPAC
  Rotterdam (NL), 9 Nov 2012

300 Participants
16 Oral presentations, 10 posters, 4 Demo

More on http://www.conffidence.eu/Project_output/workshops.php

International PhD course:

Advanced Food analysis

- Date, location

  15-19 November 2010
  Wageningen (NL)
  Organised with VLAG school

- Participants:

  60 PhD students
  6 PostDoc researchers
  from 12 countries

- Content:

  27 lectures on Food analysis by CON*ff*IDENCE members and other experts

More on http://www.conffidence.eu/Project_output/course.php

BSc education modules

- Date, location

  4 and 11 October 2011

  Dronten (NL)

  Organised with CAH Dronten University

- Participants:

  26 International students from 11 countries

  49 Dutch students

- Content:

  2 Days course on theoretical and technical issues on mycotoxins and plant toxins in food

More on http://www.conffidence.eu/Project_output/course2.php

108 Lectures and 110 posters

- International conferences

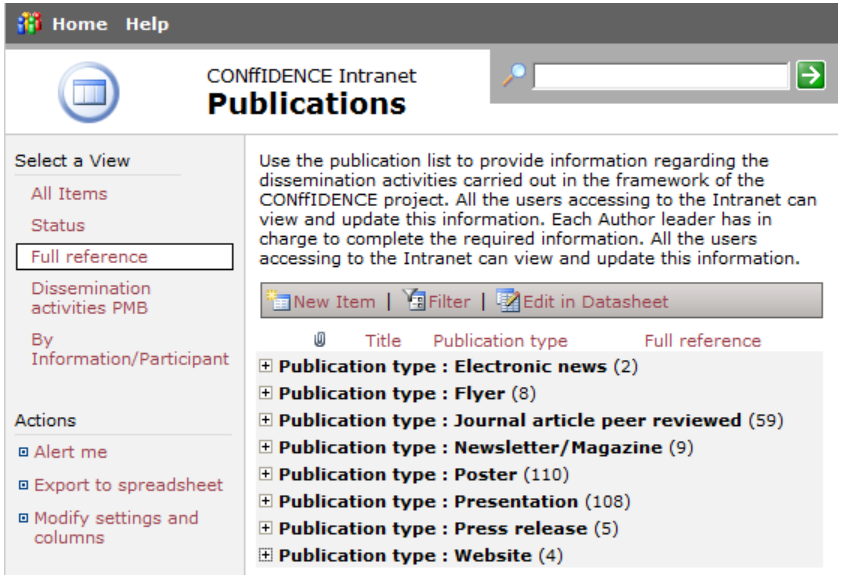
More on http://www.conffidence.eu/Project_output/posters.php

24 Publications

- Peer reviewed publications

  24 published

  35 planned



More on https://intranet.conffidence.eu/Lists/Publications/AllItems.aspx

Conclusions

- **8 e-Newsletters** have been sent to a broad public
- **3 Open Days** and **4 workshops** have been organised for the stakeholders
- **PhD Course** and **BSC education modules** have been organised for students and researchers
- Project outcomes were presented in **lectures** (**108**) and **posters** (**110**) at international conferences and as **publications** (**24**) in peer reviewed journals

Scheduled Activities 2013

- **e-Newsletter:** Special issue

  Main outputs of the project Feb 2013

- **Publications**: Special issue in ABC journal

  CON*ff*IDENCE outputs:

  4 reviews + 19 original papers

Analytical and Bioanalytical Chemistry

Editors: S. Daunert; P. Garrigues; G. Gauglitz; K.G. Heumann; K. Jinno; A. Roda; A. Sanz-Medel; S.A. Wise

Questions ?

General overview, impact and future challenges

www.conffidence.eu




SEVENTH FRAMEWORK PROGRAMME

CONFFIDENCE

Overview and impact

- ✓ Work packages are the core !
- ✓ Many useful results for different stakeholders:
  - European Commission, EU policies
  - European Food & Feed Industries (and associated laboratories)
  - EFSA
  - National governments (and associated laboratories)
  - EURL / NRL networks
  - CEN Committees for Food & Feed

European Commission

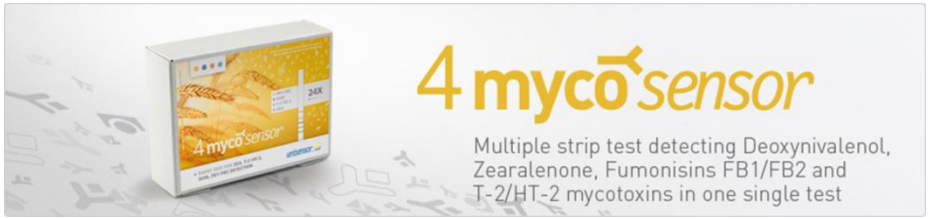
efsa European Food Safety Authority

CEN EUROPEAN COMMITTEE FOR STANDARDIZATION COMITÉ EUROPÉEN DE NORMALISATION EUROPÄISCHES KOMITEE FÜR NORMUNG

CONFIDENCE

Some special achievements (1)

- ✓ Some multi-dipsticks are already commercially available



unisensor

- ➢ Improvement of possibilities for quality control by food and feed industries
- ➢ Improvement of possibilities for official control
- ➢ Creation of jobs in Europe

Some special achievements (2)

- ✓ Many tests have been validated through small-scale collaborative studies: transferability to other laboratories has been demonstrated
- ✓ Some tests have been validated through full collaborative studies, viz. mycotoxins dipstick, inorganic arsenic, perfluorinated compounds
  - ➢ Methods can be adopted by CEN for European standardization

Some special achievements (3)

- ✓ Cross-cutting surveys, viz. fish & seafood: POPs, PFCs, heavy metals
  - Data will be available for EFSA
  - Improved exposure assessment
  - Contributions to risk-benefit (+ PUFAs)
- ✓ New topics have been included in the work programme in order to improve the relevance to EU policies (fruitful interaction with the Advisory Board!)
  - More pyrrolizidine and ergot alkaloids
  - Inorganic arsenic in rice
  - Full collaborative studies for mycotoxins and inorganic arsenic

Future challenges (1)

- ✓ Conversion of CON*ff*IDENCE methods and new technologies into fully validated methods that can be implemented. The funding of *full scale interlab studies* on methods developed in FP7 projects should be given a priority by the Commission
- ✓ For *emerging contaminants* there needs to be a system developed for calls for 'method development, validation, training' for key analytes identified by the Commission

Future challenges (2)

- ✓ Commercialization of rapid tests developed in CON*ff*IDENCE, e.g.
  - ➢ Some dipsticks
  - ➢ Kits that can be used for Luminex applications

- ✓ Development of models for risk based monitoring of contaminants in food & feed

Future challenges (3)

Stakeholder views ?