

# Cluster 4: Biotoxins



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# What Biotoxins?

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Alkaloids



Marine  
Toxins



Mycotoxins



# Why These Biotoxins?

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- Represent a substantial health risk if consumed
- Either currently legislated for or likely to be in the near future
- Either a current lack of suitable screening tests or no tests available at all.



# Alkaloids

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Pyrrolizidine alkaloids (PA)



Tropane alkaloids (TA)



Ergot alkaloids (EA)



# Objectives

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To develop three validated multiplex dipstick assays for the determination of:

- Pyrrolizidine alkaloids in honey and feed,
- Tropane alkaloids in feed
- Ergot alkaloids in feed and cereals.

To develop a validated NIR imaging method for the detection of ergot contamination in feed and food.









# Approach

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- Development of single and multiplex dipsticks for the determination of PA in honey and feed; TA in feed; and EA in feed and cereals.
- Development of Near Infra Red (NIR) spectroscopic imaging method for ergot contamination in feed and food.



# Results: first 18 months

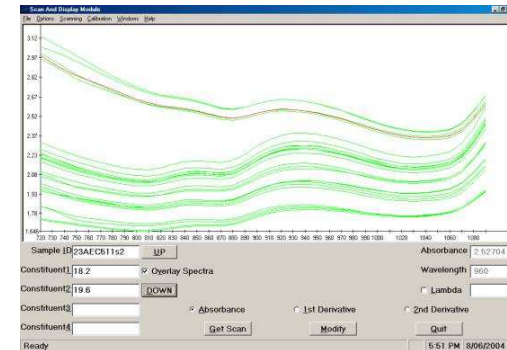
Toxins 	Test materials 	Antibodies
Jacobine	Honey (PA)	Jacobine 
Lycopsamine	Feed (all)	Lycopsamine 
Atropine	Cereal (EA)	Atropine 
Scopolamine		Scopolamine 
Ergotamine		Ergotamine
Ergocristine		Ergocristine



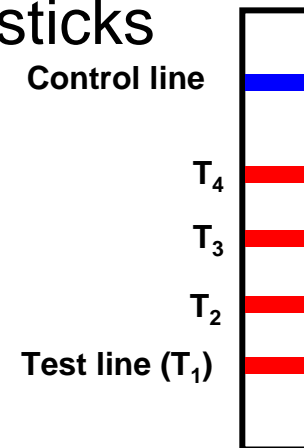
# Next 12 months

- Complete Antibodies and undertake homogeneity study test materials

- Develop Imaging method



- Develop single and multiplex dipsticks





# Marine Toxins

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## Target Toxins

Paralytic Shellfish Poisons

Diarrhetic Shellfish Poisons (OA, DTX1, DTX2)

Amnesic Shellfish Poison (Domoic Acid)

Spirolides

Palytoxins





# Objectives

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- Development of a rapid multiplex SPR biosensor assay capable of detecting a combination of major PSP and DSP toxins
- Development of SPR biosensor assays for emerging toxins, palytoxin and spirolides, to add to the multiplex assay
- Determine toxicological properties of the new emerging marine toxins, palytoxin and spirolides.














# Activities first 18 months

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- Production of toxins
  - Production of surface chemistries
  - Production of antibodies
  - Assay development
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- Toxicological assessment of spiroptides



# Results first 18 months

Toxins	Antibodies	SPR Assays
PSPs 	PSPs 	PSPs 
DSPs 	DSPs 	DSPs 
ASPs 	ASPs 	ASPs 
Spirolides 	Spirolides	Spirolides
Palytoxins 	Palytoxins	Palytoxins

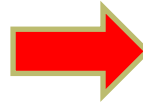
Assessment of spirolides completed and results reported to EFSA.

**Impact:** spirolides to become regulated

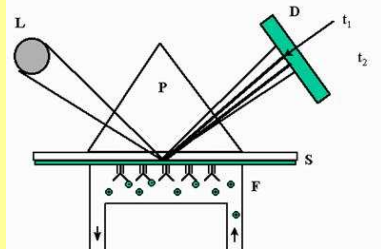


# Next 18 months: Multiplexing SPR

PSPs
DSPs
ASPs



**New Platform Technologies**



Spirolides
Palytoxins



# Mycotoxins

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To develop multiplex dipstick tests for the determination of the Fusarium toxins

DON, ZEA and T-2/HT-2 in wheat & oats  
FBs, in maize.



# Objectives and tasks

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- **Objective 1: prototype dipstick development**
  - Production and characterization of antibodies against *Fusarium* toxins
  - Dipstick design, assembly and testing with buffer solution
  
- **Objective 2: immunoassay development and validation**
  - Production and characterization of test materials
  - Development of simplified sample preparation for:  
cereals, maize-by-products for feed, cereal foods
  - Small scale interlaboratory study
  
- **Objective 3: impact demonstration**
  - On-farm mycotoxin monitoring using dipsticks





# Results first 18 months: Antibody Production

- **Zearalenone**: C50 in buffer = 0.1 ng/ml, no CR towards other mycotoxins, CR 4 - 80% towards analogue compounds ( $\alpha$ -zearanol,  $\alpha$ -zearalenol, zearalanone,  $\beta$ -zearanol,  $\beta$ -zearalenol)
- **T2** : CFSY11 showing IC50 in buffer = 2.5 ng/ml (**T-2**), no CR towards other mycotoxins except 35% for **HT-2**
- **Fumonisin B1** : CF56 showing IC50 in buffer = 0.5 ng/ml (FB1) no CR towards other mycotoxins except 364 % for **FB2** and 82% for **FB3**
- **DON** IC50 in buffer = 3 ng/ml – 190ng/ml

<i>Cross-reactivity (%)</i>	<b>M394</b>	<b>CF179</b>	<b>CF194</b>
DON	100	100	100
3-acetyl DON	180	311	614
15-acetyl DON	6	-	-
DON glucoside	ND	50	118
Nivalenol	-	9	44
Fusarenon X	-	8	99

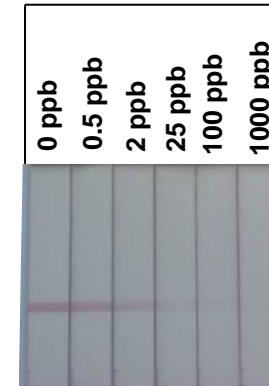


# Results: prototype dipstick development

Single dipsticks in spiked buffer for  
FB1, ZEA, T2 and DON  
(Incubation 5 min. - Migration of 5 min. @ RT)



## Zearalenone



**IC50 = 2ppb**

## Fumonisin B1



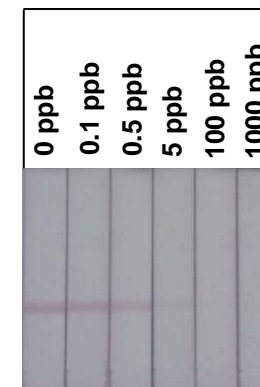
**IC50 = 5ppb**

## DON



**IC50 = 5ppb**

## T2



**IC50 = 1ppb**



# Ultimate Objective

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- Constructed dipstick immunoassays for cereals, cereal based foods and feed are expected to be available within the 3<sup>rd</sup> project year

## Activities for the 4<sup>th</sup> project year:

- Validation of dipstick immunoassays through small scale interlaboratory study involving all WP4c partners.
- Impact demonstration: on farm testing with the aim of drafting a monitoring plan for mycotoxins based on the use of multiplex dipsticks.



# Summary

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A wide range of activities being performed in the biotoxin cluster

Working towards rapid, reliable screening assays to support regulatory and industrial monitoring programmes

For further information contact co-ordinator and visit project website

