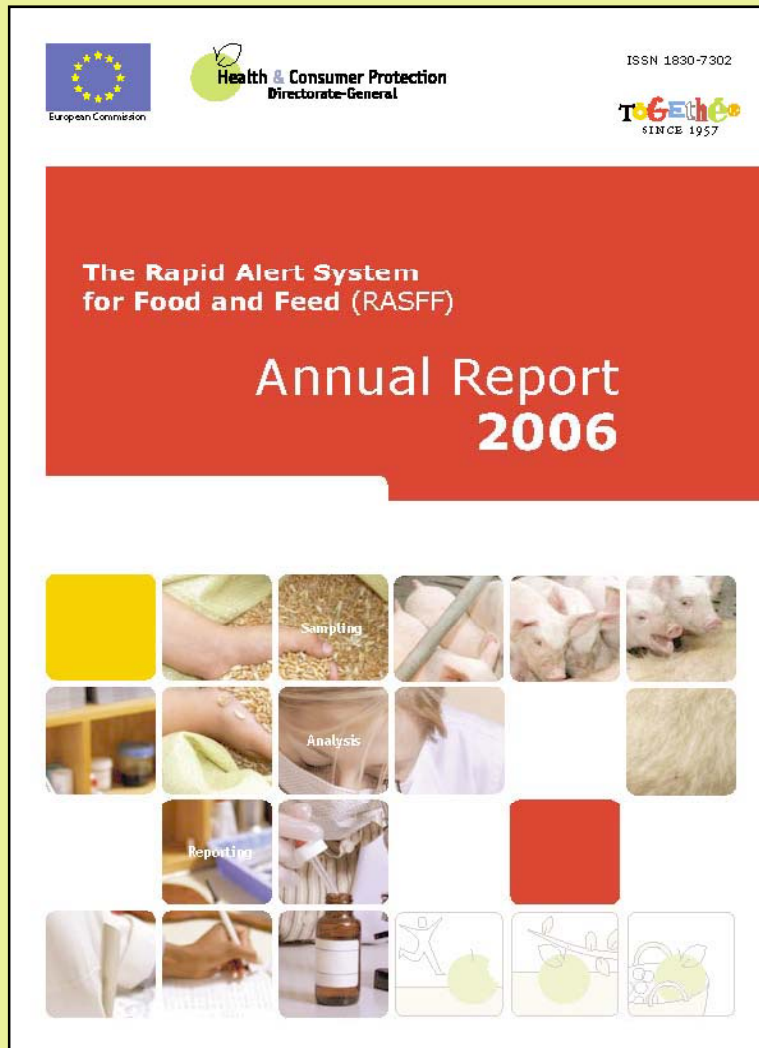


Mycotoxins in Europe: CEN activities and the EU-projects

Hans van Egmond - RIVM Bilthoven

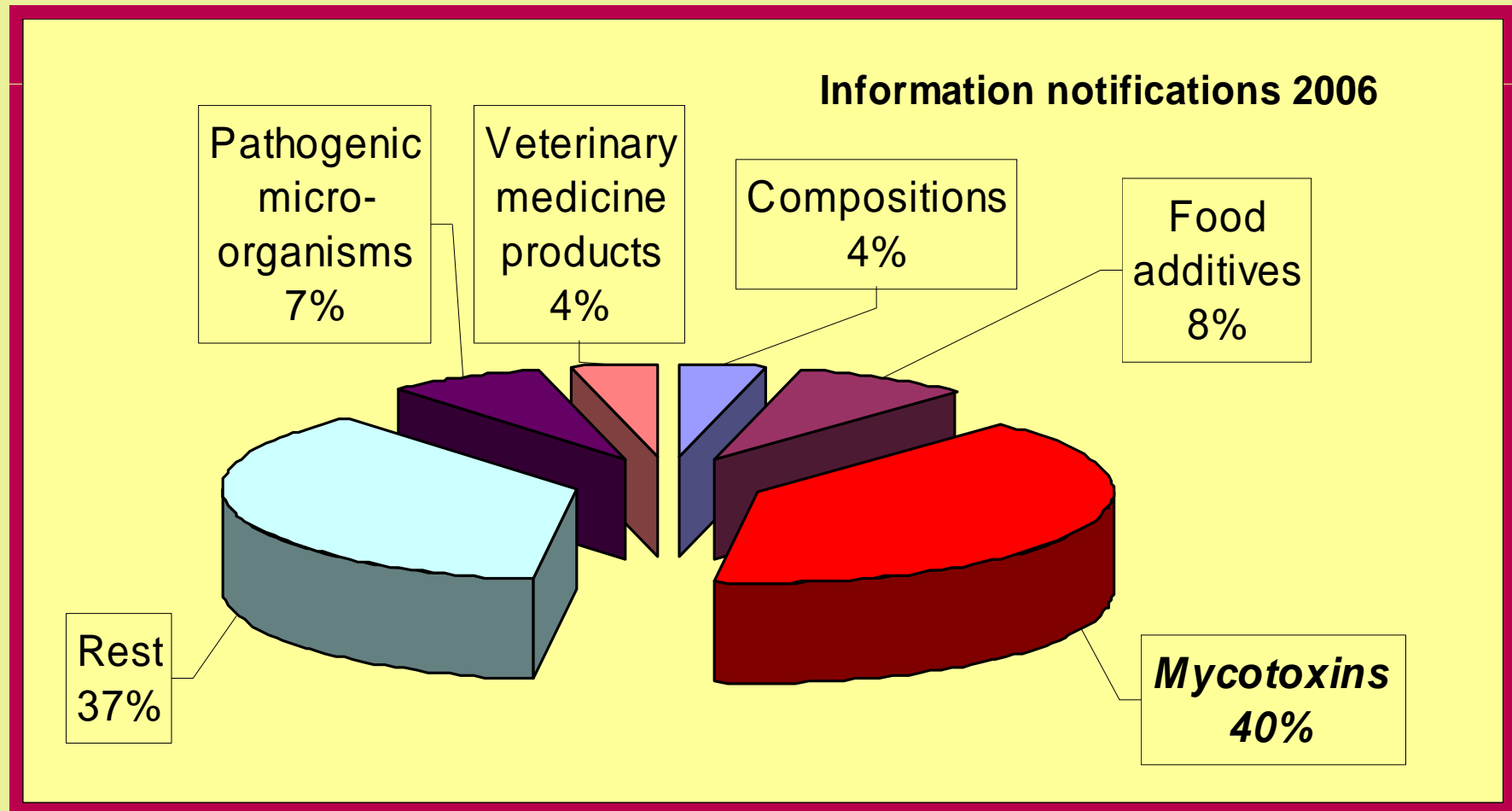
European concern about mycotoxins



RASFF:

- Quick information-exchange in the EU on risks to human health
- Allows MS to identify potential problems and take measures
- In 2006: 800 mycotoxin issues

RASFF 2006: information notifications



Europe's interest in analytical projects on natural toxins



- Standardization of methods for **mycotoxins** and phycotoxins in Europe since the 1990's



- FP6: projects including analytical methods for **mycotoxins** and phycotoxins: e.g. **BioCop, MoniQA**



- FP7: projects including analytical methods for **mycotoxins**, phycotoxins and plant toxins, e.g. **Confidence**

Europe's interest in analytical projects on natural toxins



- Standardization of methods for mycotoxins and phycotoxins in Europe since the 1990's



- FP6: projects including analytical methods for mycotoxins and phycotoxins: e.g. **BioCop**, **MoniQA**



- FP7: projects including analytical methods for mycotoxins, phycotoxins and plant toxins, e.g. **Confidence**




- CEN = Comité Européen de Normalisation (European Committee for Standardization)
- Members: European countries, not exclusively the European Union
- Technical Committees / working groups
- TC 275: “Food analysis–Horizontal methods”

What is a CEN standard?

- Public document, “agreement”, prepared by consensus
- Made for and by the market
- National Standard Bureaus facilitate the process and deliver the end product
- Status of a standard is determined by the use (e.g. reference in contract, legislation)



CEN standardizes mycotoxin methods

- ★ • **Mycotoxins** (and phycotoxins) group active since early 1990s, meetings usually once a year
- ★ • Chair and secretariat:  The Netherlands
- ★ • CEN WG 5 standardizes *quantitative* (bio-) chemical methods for biotoxins, no bioassays
- ★ • Selection based on method performance, in line with EU legislative approach for mycotoxins



EU legislation on mycotoxins

- Now exists for approx. 70 toxin-matrix combinations
- Does not specify methods of analysis
- Allows the use of analysis methods that fulfill certain performance criteria
- Gives a prominent role to CEN, because CEN
 - develops these performance criteria
 - standardizes methods that fulfill to these criteria

Current and coming CEN mycotoxin methods



- Sofar all mycotoxin methods based on chromatographic techniques
- Future methods could include other techniques (e.g. sensors)
- Selected on the basis of interlaboratory performance
- CEN methods now available for 10 mycotoxin- matrix combinations
- CEN interacts with EU projects

Example of front page of CEN standard

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 14123

June 2003



ICS 67.050

English version

Foodstuffs - Determination of aflatoxin B₁ and the sum of aflatoxin B₁, B₂, G₁ and G₂ in peanuts, pistachios, figs, and paprika powder - High performance liquid chromatographic method with postcolumn derivatization and immunoaffinity column clean-up

EC mandate addressed to CEN (2007)

- Establish standardized mycotoxin methods to enable compliance with EU legislation in a harmonized way
- Methods included for PAT, DON, ZEN, AFL B₁, OTA & FUM in various specified foods, second mandate in preparation
- Review and revise performance criteria for methods of analysis of mycotoxins



Mandated review of performance criteria for mycotoxin methods



- Target: technical report that specifies criteria for the selection of methods of analysis
- Criteria cover repeatability, reproducibility, recovery, extraction solvents, applicability
- Performance criteria are included for 10 different toxins or toxin groups, of EU legislative relevance
- Report to contain information on terms and definitions, validation, standardization procedures and interlaboratory studies

Europe's interest in analytical projects on natural toxins



- Standardization of methods for mycotoxins and phycotoxins in Europe since the 1990's



- FP6: projects including analytical methods for mycotoxins and phycotoxins: e.g. **BioCop**, **MoniQA**

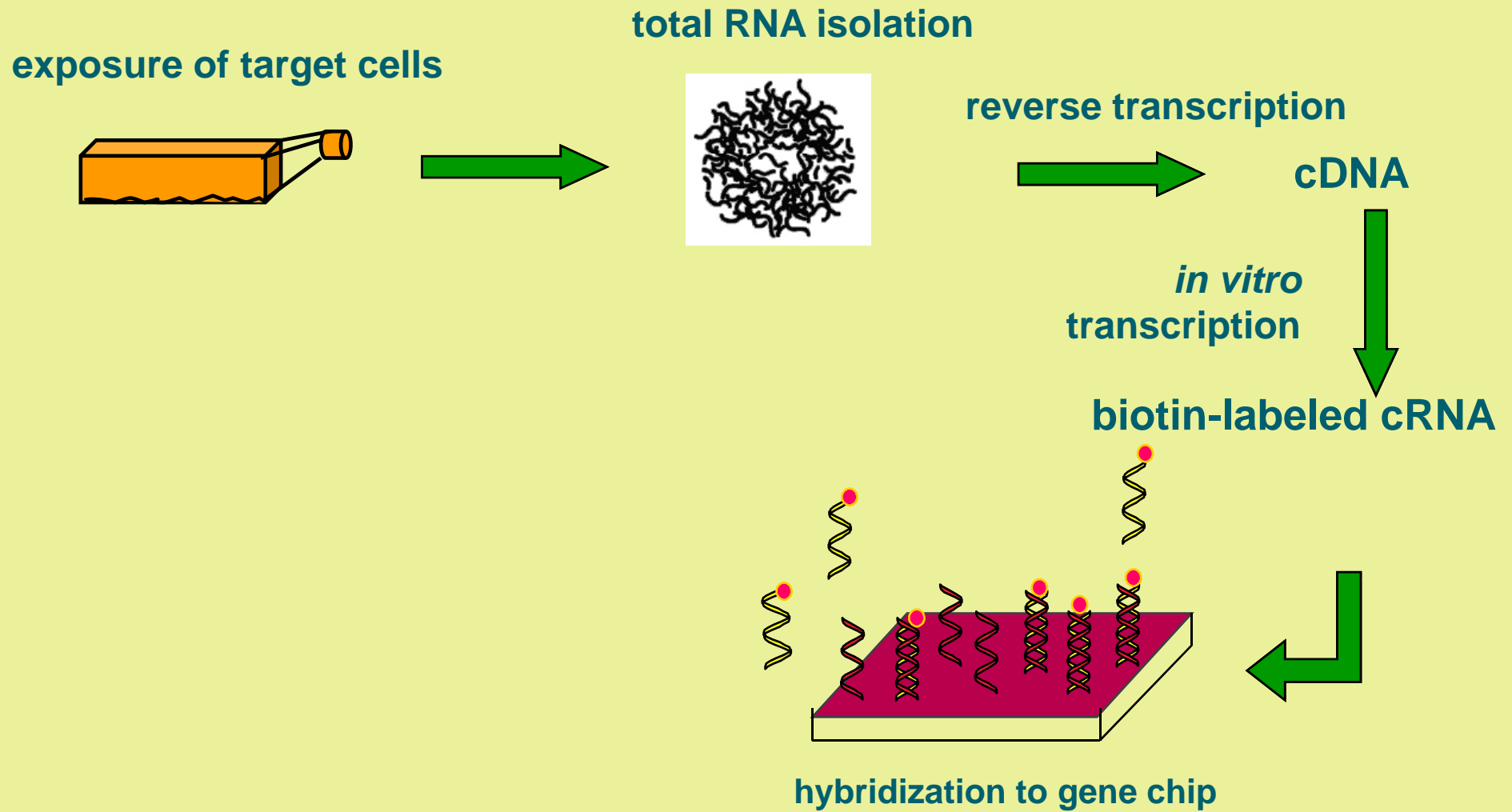


- FP7: projects including analytical methods for mycotoxins, phycotoxins and plant toxins, e.g. **Confidence**

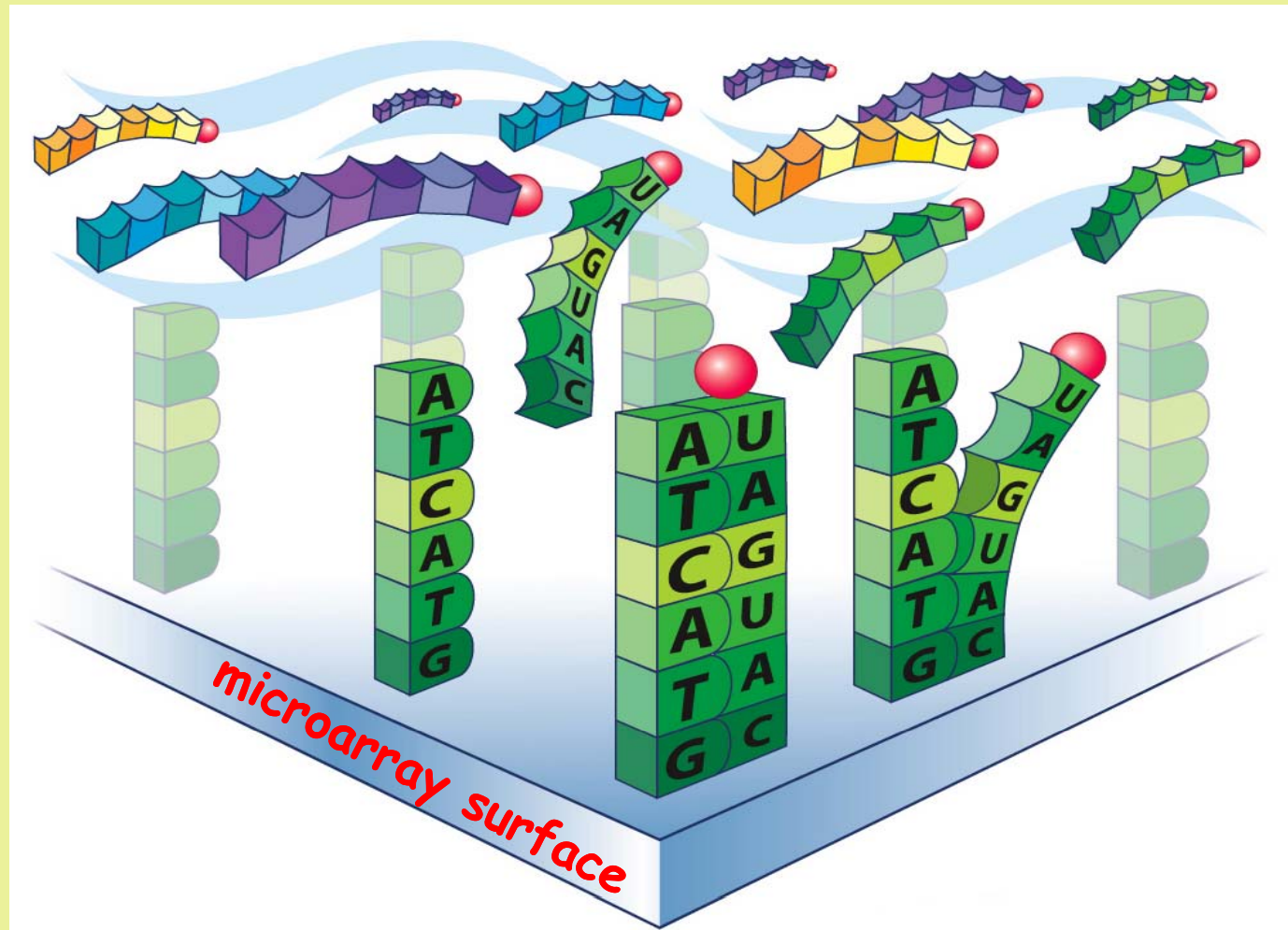


- **IP** Food Quality & Safety, 2005-2010
- New biotech-based methods explored and exploited
- Involves various analyte groups
- Includes WP on **trichothecenes** (electrochemical & SPR sensors and transcriptomics)

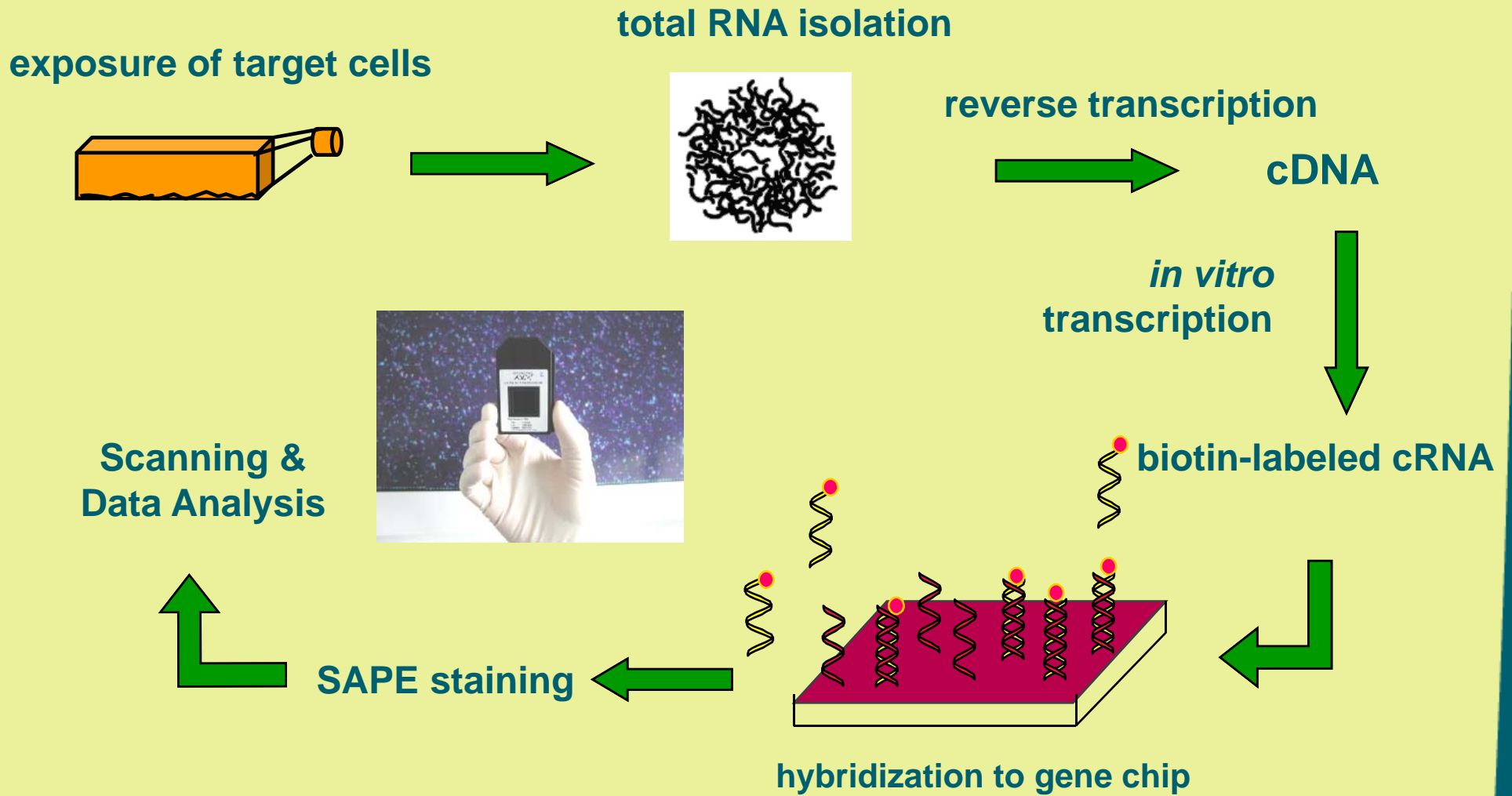
Transcriptomics: assay



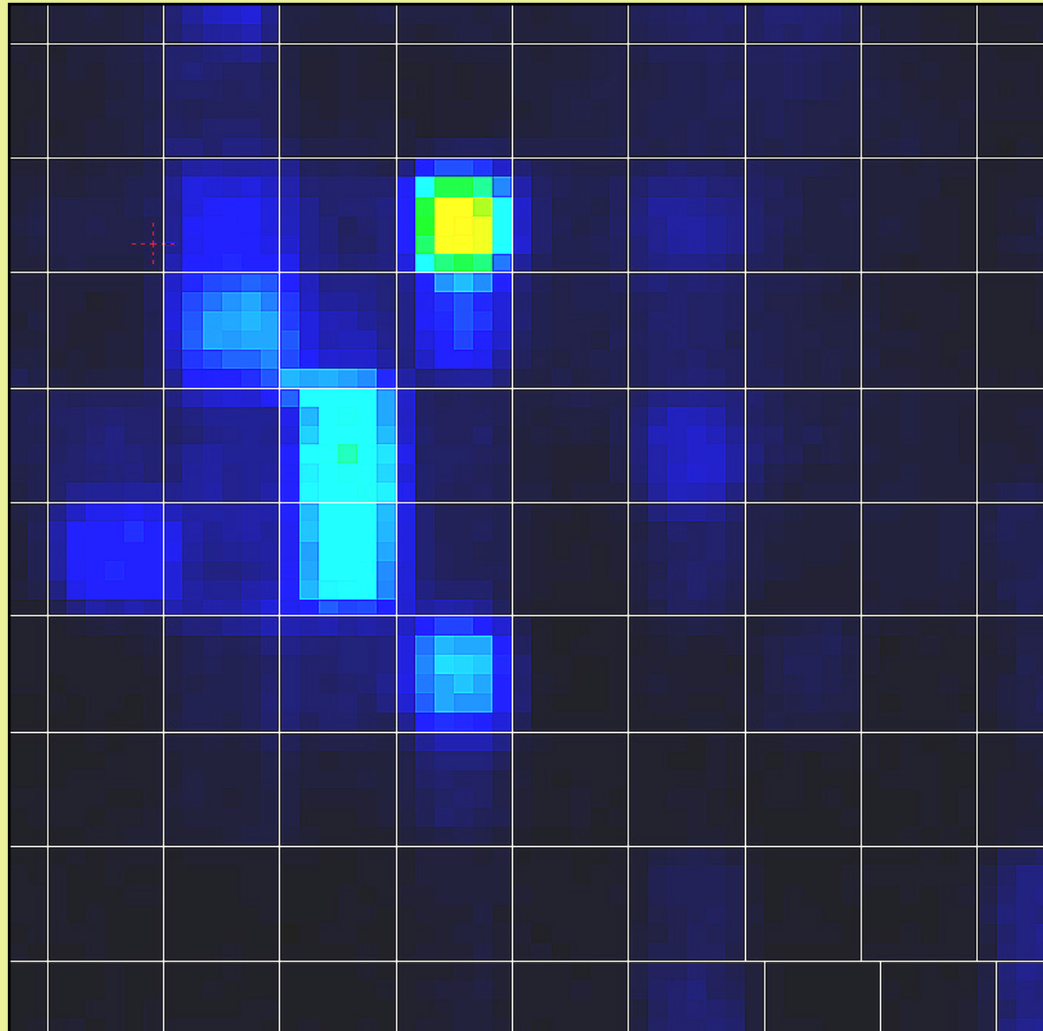
Transcriptomics: hybridization



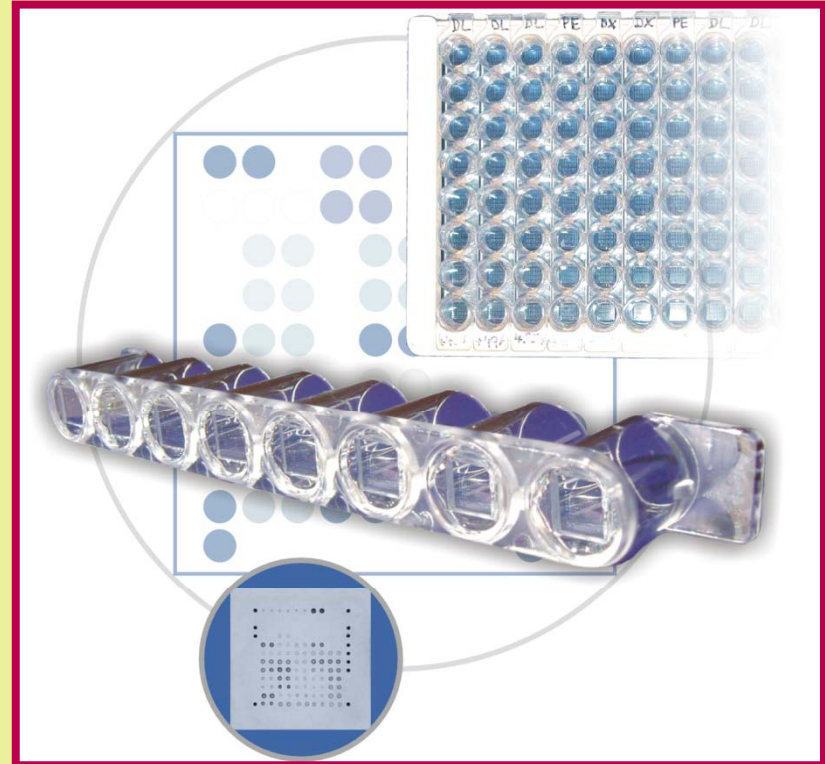
Transcriptomics: assay



Transcriptomics: visualization of binding



New development: cup/strip/plate with chips

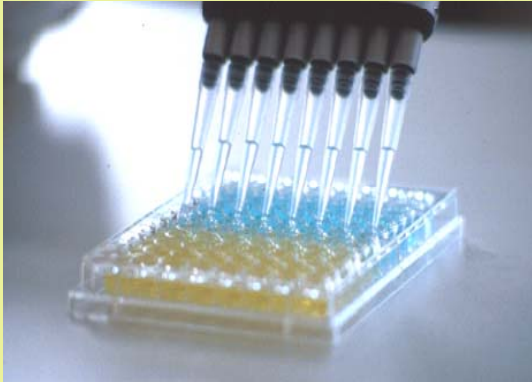


MoniQA



- **NoE** Food Quality & Safety, 2007-2011
- Focus on harmonization of analytical methods to monitor food hazards
- Integration, joint research, spreading of excellence
- Includes WG **mycotoxins** and phycotoxins
- Emphasis on rapid methods

Rapid method format



- Immunochemical detection methods
 - ELISA
 - Dipstick Assay/Lateral Flow Device
 - Fluorescence Polarisation
 - Biosensors/Lab on a chip



- DNA-based methods
 - PCR + gel electrophoresis
 - PCR-ELISA
 - Real-Time PCR
 - Biosensors/Lab on a chip

Europe's interest in analytical projects on natural toxins



- Standardization of methods for mycotoxins and phycotoxins in Europe since the 1990's



- FP6: projects including analytical methods for mycotoxins and phycotoxins: e.g. **BioCop**, **MoniQA**



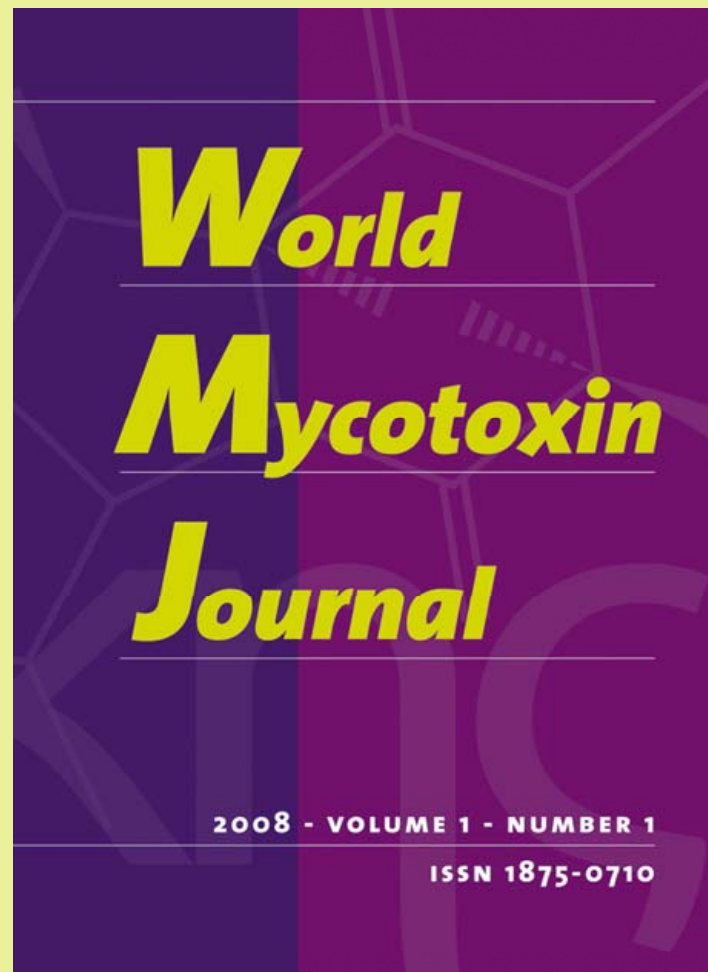
- FP7: projects including analytical methods for mycotoxins, phycotoxins and plant toxins, e.g. **Confidence**



- **LCP** Food, Agriculture and Fisheries, and Biotechnology, 2008-2012
- Simple, fast, multi-analyte, multi-class detection
- Training workshops and educating modules
- Includes WP Biotoxins
 - alkaloids (PA, tropane, **ergot**)
 - *Fusarium* **mycotoxins**
 - phycotoxins

2008: launch of *World Mycotoxin Journal*

- European initiative with global participation
- Editorial board includes representatives from:
 - FAO, ICC, (IARC)
 - EFSA, EC-DG SANCO, JRC-IRMM
 - EU-project partners
 - 6 continents, 20 countries



Coming mycotoxin events in Europe, 2008 and 2009



- World Mycotoxin Forum
(celebrating its 5th anniversary)
17-18 November 2008, Noordwijk, The Netherlands
- ISM conference 2009
(International Society for Mycotoxicology)
9-11 September 2009, Tulln/Vienna, Austria

Thank you for your attention

- 'All parties concerned'
- Consensus principle



- Disadvantage: development time
- Advantage: result broadly accepted



Table of contents of a CEN standard

Foreword

1 Scope

2 Normative references

3 Principle

4 Reagents

5 Apparatus

6 Sampling

7 Procedure

8 Calculation

9 Precision

10 Test report

Annex A (informative) – Precision data

Annex B (informative) – Typical chromatogram

Bibliography



CEN w.g. mycotoxins

Standardized aflatoxin methods

- AFL B₁ and total AFL in cereals, shell fruits and derived products, IA clean-up, LC, PCD + FLD
- AFL B₁ and total AFL in peanuts, pistachios, figs, and paprika powder, IA clean-up, LC, PCD + FLD

CEN w.g. mycotoxins

Standardized ochratoxin A methods

- OTA in cereals and cereal products, silica gel clean-up, LC-FLD
- OTA in cereals and cereal products, bicarbonate clean-up, LC-FLD
- OTA in barley and roasted coffee, IA clean-up, LC-FLD
- OTA in wine and beer, IA clean-up, LC-FLD

CEN w.g. mycotoxins

Standardized fumonisin methods

- FUM B₁ and B₂ in maize, SAX clean-up, OPA derivatization, LC-FLD
- FUM B₁ and B₂ in maize-based foods, IA clean-up OPA derivatization, LC-FLD

CEN w.g. mycotoxins

Standardized patulin method

- PAT in clear and cloudy apple juice, liquid-liquid extraction, LC-UV

What is on the horizon?

- Patulin in food for infants and young children
- DON in cereals, baby food and feed
- OTA in dried fruit, baby- & dietary foods and cocoa
- Aflatoxins in hazelnut paste and in food for infants and young children
- ZEA in cereals, baby food and feed
- Fumonisin in baby food and feed
- T-2 and HT-2 toxins in cereals, baby food and feed
- Ergot alkaloids in cereals and feedstuffs
- Update and upgrade of criteria document