# New tests keep poisons out of food—and off the table

#### Validated RNRRS Output.

A simple and affordable diagnostic tool is allowing food companies to measure the mycotoxin content of their foods. Mycotoxins are highly poisonous compounds produced by certain moulds that grow on a wide variety of foods and feeds. When eaten, they can cause disease and even death in livestock and people. Mycotoxin ingestion causes about 250,000 deaths a year in parts of sub-Saharan Africa. In cereals, edible nuts and oilseeds, the distribution of mycotoxins is highly localised. The new technology addresses this problem, zeroing in on infested areas accurately. This will have a major impact on food safety and productivity, significantly reducing the costs of testing. What is more important, it is available to all players, including people in developing countries with limited resources.

Project Ref: **CPH17:** Topic: **5. Rural Development Boosters: Improved Marketing, Processing & Storage** Lead Organisation: **Natural Resources Institute (NRI), UK** Source: **Crop Post Harvest Programme** 

**Document Contents:** 

Description, Validation, Current Situation, Environmental Impact,

## Description

CPH17

**Research into Use** 

NR International Park House Bradbourne Lane Aylesford Kent ME20 6SN UK

Geographical regions included:

Africa, UK,

Target Audiences for this content:

Livestock farmers, Consumers,

RIU

#### A. Description of the research output(s)

1. Working title of output or cluster of outputs.

In addition, you are free to suggest a shorter more imaginative working title/acronym of 20 words or less.

#### The development of technologies for the control of mycotoxins in human food and livestock feed

2. Name of relevant RNRRS Programme(s) commissioning supporting research and also indicate other funding sources, if applicable.

#### Crop Post Harvest Programme University of Greenwich Higher Education Funds

3. Provide relevant R numbers (and/or programme development/dissemination reference numbers covering supporting research) along with the institutional partners (with individual contact persons (if appropriate)) involved in the project activities. As with the question above, this is primarily to allow for the legacy of the RNRRS to be acknowledged during the RIUP activities.

**Current contact:** Dr John Orchard, Natural Resources Institute, University of Greenwich, Central Avenue, Chatham, Kent ME4 4TB. Tel: 01634 883741; Fax 01634 880077. E-mail J.E.Orchard@gre.ac.uk

#### R5898 – Development of a second generation biosensor for the detection of mycotoxins

Natural Resources Institute, University of Greenwich: Dr Raymond Coker and Martin Nagler School of Science, University of Greenwich: Prof. E Metcalfe, Prof. A Tseung and Dr Rashid Deane

#### R6125 - The development of sampling plans for the determination of aflatoxins in feeds

NRI: Dr Raymond Coker, Martin Nagler, John Gibbs RIKILT-DLO, The Netherlands TNO Centre for Applied Statistics (TNO-TPD), The Netherlands University of Hamburg, Germany

#### R6127 – Rapid methods for the analysis of mycotoxins

NRI: Dr Raymond Coker, Martin Nagler University of Portsmouth: Professor Gerald Blunden

#### R6091 – Yeast bioassay for the detection of mycotoxins

NRI: Dr Raymond Coker University of Greenwich: Dr Ivor Evans

4. Describe the RNRRS output or cluster of outputs being proposed and when was it produced? (max. 400 words).

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RESEARCH INTO USE PROGRAMME: RNRRS OUTPUT PROFORMA
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This requires a clear and concise description of the output(s) and the problem the output(s) aimed to address. Please incorporate and highlight (in bold) key words that would/could be used to select your output when held in a database.

The cluster of outputs was produced during the period 1993 – 1997 as a contribution towards the development of a strategy to control the occurrence of **mycotoxins** in foods.

Mycotoxins are highly poisonous compounds which are produced by certain **moulds** when they grow on a wide variety of **foods and feeds**. The ingestion of mycotoxins by humans or livestock may cause **disease**, **decreased productivity and death**, and their occurrence in foods and feeds is strictly controlled by both international and national legislation. About 250,000 hepatocellular carcinoma-related deaths occur annually in parts of sub-Saharan Africa due to aflatoxin ingestion. Clearly, technologies are required for the cost-effective **detection and measurement** of mycotoxins, so that procedures for the **prevention and removal (cure)** of these compounds from foods and feeds can be developed and implemented.

Since the **distribution** of some mycotoxins (especially **aflatoxins**) in granular foods and feeds (e.g. **edible nuts**, **oilseeds and cereals**) is highly localised, **sampling plans** are required that accommodate this distribution pattern, and which enable the collection of samples which accurately represent the batch which is being evaluated.

Once a **representative sample** has been collected, technologies are then required which allow the presence of toxins to be detected and accurately measured. These technologies, involving the **detection and measurement of mycotoxins**, can be used as a means of segregating uncontaminated and contaminated commodities, and as a means of monitoring the development of effective preventative and curative measures.

It is essential that **food safety** management procedures are transparent and effective. If they are to be effectively applied from the **farm to the fork**, it is also essential that they are available to all players within the **global food system**, including those with limited resources within developing countries.

**Project R6125** produced simple but effective sampling plans for the accurate sampling of **large shipments** (e.g. 15 – 50,000 tonnes) of livestock feeds on arrival at European ports, prior to **aflatoxin** analysis.

**Project R6091** afforded a simple **yeast bioassay** for the detection of mycotoxins, and other toxins, in foods and feeds. The technology exploited the visual impact of mycotoxins, via a colorimetric end-point, on a yeast (*Kluyveromyces marxianus*) contained within a micro-titre plate. The bioassay was applied to a variety of mycotoxins (aflatoxin B<sub>1</sub>; and thirteen mycotoxins).

**Project R5898** initiated the development of a **biosensor**, which exploited an anti-aflatoxin  $B_1$  antibody, for the measurement of mycotoxins in selected foods and feeds.

**Project R6127** initiated the development of a **simple and rapid** method for the accurate measurement of selected mycotoxins using **mini-column** technology using a specially designed fluorimeter.

5. What is the type of output(s) being described here? Please tick one or more of the following options.

Product	Technology	 Process or Methodology	Other Please specify
	Х	Х	

6. What is the main commodity (ies) upon which the output(s) focussed? Could this output be applied to other commodities, if so, please comment

The targeted commodities were:

**R6125** - copra meal pellets, copra cake and palm kernel cake. In principle, the sampling plans developed could be applied to any aflatoxin-contaminated *processed* commodity where the processing involved the crushing or comminution of granular material (e.g. oilseed cakes and meals, cereal flours).

R6091- groundnuts and corn (maize)

R5898 - groundnuts

R6127- groundnuts and corn (maize)

In principle, the outputs generated by projects R6091, R5898 and R6127 can be applied to a variety of commodities (e.g. edible nuts and cereals), providing the toxin can be effectively extracted and any potentially interfering material can be effectively removed before the assay is performed.

7. What production system(s) does/could the output(s) focus upon? Please tick one or more of the following options. Leave blank if not applicable

Semi-Arid	High potential			 Tropical moist forest	Cross- cutting
Х	Х	X	Х		

8. What farming system(s) does the output(s) focus upon?

Please tick one or more of the following options (see Annex B for definitions). Leave blank if not applicable

Smallholder rainfed humid	<b>J</b>		Smallholder rainfed highland		Coastal artisanal
			U	•	fishing
Х	Х	Х		Х	

9. How could value be added to the output or additional constraints faced by poor people addressed by clustering this output with research outputs from other sources (RNRRS and non RNRRS)? (**max. 300 words**).

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Please specify what other outputs your output(s) could be clustered. At this point you should make reference to the circulated list of RNRRS outputs for which proformas are currently being prepared.

The global food chain is becoming increasingly regulated and, within this scenario, more powerful players (e.g. supermarkets and their larger suppliers) are pushing responsibility for food safety management back up the chain towards the smaller players, including those in developing countries. Similarly, the introduction of modern food safety management methods which focus upon the control of the process (e.g. Hazard Analysis and Critical Control Points, HACCP), from the farm to the fork, require the involvement of all players, including those with limited resources.

Consequently, it is essential that the smaller players in the food chain (whether domestic or global) are armed with cost-effective procedures for the management of the safety of their food and feed products.

The control of mycotoxins in foods and feeds is particularly difficult because uncontrollable climatic conditions can lead to mycotoxin contamination before harvest and immediately after harvest (if the producer depends upon sundrying); and mycotoxins in foods and feeds are normally highly heat-stable once contamination has occurred. Consequently, the effective control of mycotoxins remains significantly dependent upon segregation procedures, even when preventative control measures are in place; and, in turn, there is an urgent demand for cost-effective and transparently efficient sampling and analysis methods which can be employed throughout the food chain to facilitate the segregation of 'good' and 'bad' material (R7809: Strategies for reducing aflatoxin levels in groundnut based foods and feeds in India and NRI project: Control of mycotoxins in cereal production and processing chains in Latin America).

The types of sampling and analysis methods should be clustered with other outputs which address the management of food safety within domestic and global markets; including domestic markets (NRI project on Training the Trainers to Implement Food Safety Improvement Programmes in African Food Production and Processing)

### Validation

#### B. Validation of the research output(s)

10. How were the output(s) validated and who validated them?

Please provide brief description of method(s) used and consider application, replication, adaptation and/or adoption in the context of any partner organisation and user groups involved. In addressing the "who" component detail which group(s) did the validation e.g. end users, intermediary organisation, government department, aid organisation, private company etc... This section should also be used to detail, if applicable, to which social group, gender, income category the validation was applied and any increases in productivity observed during validation (**max. 500 words**).

#### R6125– The development of sampling plans for the determination of aflatoxins in feeds

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The outputs (sampling plans) were developed and validated by the TNO Centre for Applied Statistics (TNO-TPD), The Netherlands. Samples were collected at Rotterdam Docks by RIKILT-DLO, The Netherlands and at Immingham, UK by the NRI. The aflatoxin analyses were performed by the NRI and the University of Hamburg, employing standard quality control procedures. The outputs were also discussed in detail, at a workshop in Brussels, with representatives of the EU (DGVI and DGXII) and The Royal Dutch Grain and Feed Trade Association. Finally, the project outputs were subjected to peer review and published in the *Journal of the AOAC International* **83**(5), 1252-1258 (2000) – Sampling Plans for the Determination of Aflatoxin B<sub>1</sub> in Large Shipments

of Animal Feedstuffs.

#### R6127 – Rapid methods for the analysis of mycotoxins

The rapid methods developed during this project were validated by the project team using standard procedures, including their comparison with reference high performance liquid chromatography (HPLC) procedures. The project outputs were subjected to peer review and successfully published as a PhD thesis: *The Development and Validation of Rapid, Robust, Low-cost Methods for the Determination of Mycotoxins in Cereals*; Stephen Yeo, University of Portsmouth, 1999.

#### R6091 – Yeast bioassay for the detection of mycotoxins

The yeast bioassay developed during this project was evaluated by the project team by challenging the bioassay with a variety of mycotoxins, including aflatoxin  $B_1$  and thirteen trichothecene mycotoxins. The procedure

provided a sensitive method for the detection of the trichothecene mycotoxins and was used to successfully determine the structure-activity relationships within this group of toxins. The project outputs were also subjected to peer review and successfully published as a PhD thesis: Development and Assessment of a Yeast Bioassay for the Detection of Mycotoxins; Kathryn Engler, University of Greenwich, 1996; and in *Biotechnology Letters* **22**, 3-8 (2000) – Toxin-binding properties of cytochrome P450 in <u>Saccharomyces cerevisiae</u> and <u>Kluyveromyces</u> <u>marxianus;</u> and in *Arch. Microbiol.* **174**, 381-385 (2000) – Uptake of aflatoxin B<sub>1</sub> and T-2 toxin by two mycotoxin

bioassay microorganisms: Kluyveromyces marxianus and Bacillus megaterium.

#### 11. Where and when have the output(s) been validated?

Please indicate the places(s) and country(ies), any particular social group targeted and also indicate in which production system and farming system, using the options provided in questions 7 and 8 respectively, above (max 300 words).

The outputs been validated within the laboratory using accepted methods but they remain to tested in production and farming systems.

# **Current Situation**

#### C. Current situation

12. How and by whom are the outputs currently being used? Please give a brief description (max. 250 words).

#### R5898 - Development of a second generation biosensor for the detection of mycotoxins

The development programme initiated by this output was successfully exploited by a further project which developed a prototype biosensor employing a direct competitive Enzyme Linked Immunosorbent Assay (ELISA) to perform the molecular recognition between the analyte (aflatoxin B<sub>1</sub>) and its antibody, at the surface of a screen printed electrode (SCE).

#### R6127 – Rapid methods for the analysis of mycotoxins

The development and commercialisation of this output has been continued by Professor Coker using commercial funding from a variety of sources.

Four prototype instruments have been successfully manufactured and validated, and appropriate cartridges (as opposed to mini-columns) for the immobilisation of the aflatoxins and ochratoxin A have been successfully produced and evaluated. Currently, the technology is being further developed to facilitate the simultaneous measurement of several toxins, within a given sample, without the need for chemical separation.

Extensive market research, within a wide variety of food companies, has demonstrated a clear need for the product under development (the Toximet T System), the need being driven by existing and planned EC regulations specifying MRLs for a variety of mycotoxins in a broad range of commodities.

#### R6091 – Yeast bioassay for the detection of mycotoxins

The outputs of this project have been further developed by the activities of two University of Greenwich research projects E0022 (Yeast bioassays for mycotoxins and other toxins) and C1667 (Development of bioassay for determination of food toxins). A chromogenic yeast bioassay was developed, especially for those toxins requiring metabolic activation, through the genetic engineering of strains of *Saccharomyces cerevisiae*. The outputs were subjected to peer review and successfully published as a PhD thesis – A Novel, Genetically Engineered , Yeast-based Bioassay; Xingmin Li, University of Greenwich, 2005.

13. Where are the outputs currently being used? As with Question 11 please indicate place(s) and countries where the outputs are being used (max. 250 words).

#### R6127 (X0273) – Rapid methods for the analysis of mycotoxins

As described above, the outputs are currently being exploited by Toximet Limited, a planned University of Greenwich spin-out company, located at the Medway Enterprise Hub, Chatham Maritime.

14. What is the scale of current use? Indicating how quickly use was established and whether usage is still spreading

(max 250 words).

#### R6127 (X0273) - Rapid methods for the analysis of mycotoxins

Toximet Limited plans to market the Toximet T System in some twenty countries over the next four years focusing, in the first instance, on the control of mycotoxins in edible nuts, oilseeds, cereals, dried fruit, spices and coffee.

15. In your experience what programmes, platforms, policy, institutional structures exist that have assisted with the promotion and/or adoption of the output(s) proposed here and in terms of capacity strengthening what do you see as the key facts of success? (max 350 words).

All companies within the global food chain have to meet food safety regulations (e.g. Codex, EU, national) in order to retain their most lucrative markets. Consequently, they have to ensure that excessive levels of poisonous chemicals, from pesticides, antibiotics and hormones, to naturally-occurring poisons such as mycotoxins, do not find their way into foods.

Extensive market research has shown a high demand for a simple and affordable food diagnostic tool that enables food companies to readily measure the toxin content of their foods.

Toximet has exploited new technology to develop a novel tool, the Toximet T System, which provides a simple and affordable means of measuring toxins in food. Initially, the purpose of the Toximet T System is to accurately measure the levels of mycotoxins present in food raw materials and finished products, in order to reduce the impact of these potent food hazards on human and animal health, and to satisfy regulatory and trade specifications. Toximet proposes a fundamental shift in the rules of market engagement in the testing of food within a rapidly growing market. It will empower all food companies to meet the requirements of a heavily and increasingly regulated sector by performing their own tests, rather than employing the expensive services of contract analysts.

Toximet aims to be the global leader in food diagnostic systems and, consequently, to have a major impact on food safety and productivity, and to significantly reduce the costs of testing, throughout the global food chain.

From its third year of operation, Toximet will also develop and sell non-mycotoxin applications in other niche markets, both within and outside of the food sector. Additional poisons that are currently of concern to the food sector include undesirable levels of pesticide residues (in fruit and vegetables products), veterinary drugs (in fish, meat and honey), toxic compounds produced during processing and cooking (e.g. chloropropanols and acrylamide), illegal additives (e.g. Sudan 1 and Para Red dyes), algal toxins (shell-fish poisons) and food allergens.

# **Environmental Impact**

#### H. Environmental impact

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24. What are the direct and indirect environmental benefits related to the output(s) and their outcome(s)? (max 300 words)

This could include direct benefits from the application of the technology or policy action with local governments or multinational agencies to create environmentally sound policies or programmes. Any supporting and appropriate evidence can be provided in the form of an annex.

The outputs from project R6127 (and, subsequently, C1679) have produced a technology platform which, potentially, can be used for the accurate measurement of a wide range of analytes, including environmental hazards.

Similarly, the outputs from project R6091 (and, subsequently, E0022), after further development, will make an important contribution towards the understanding of the toxicology of environmental hazards, and of combinations of these and other hazards.

Clearly, the successful development and exploitation of simple, inexpensive technology for the determination and understanding of environmental hazards will have a direct positive impact upon the environment.

25. Are there any adverse environmental impacts related to the output(s) and their outcome(s)? (max 100 words)

There are no adverse environmental impacts related to the outputs and outcomes described above.

26. Do the outputs increase the capacity of poor people to cope with the effects of climate change, reduce the risks of natural disasters and increase their resilience? (max 200 words)

The outputs and outcomes, if successfully applied within the food chain, will result in the ingestion of safer food. This, in turn, will lead to healthier, more resilient populations who are better placed to cope with the effects of climate change.