

Government Chemist Programme

Referee Cases Update

Michael Walker

21 June 2016

Science
for a safer world





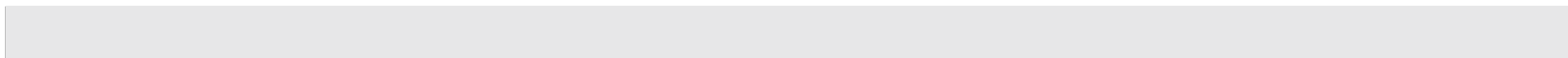
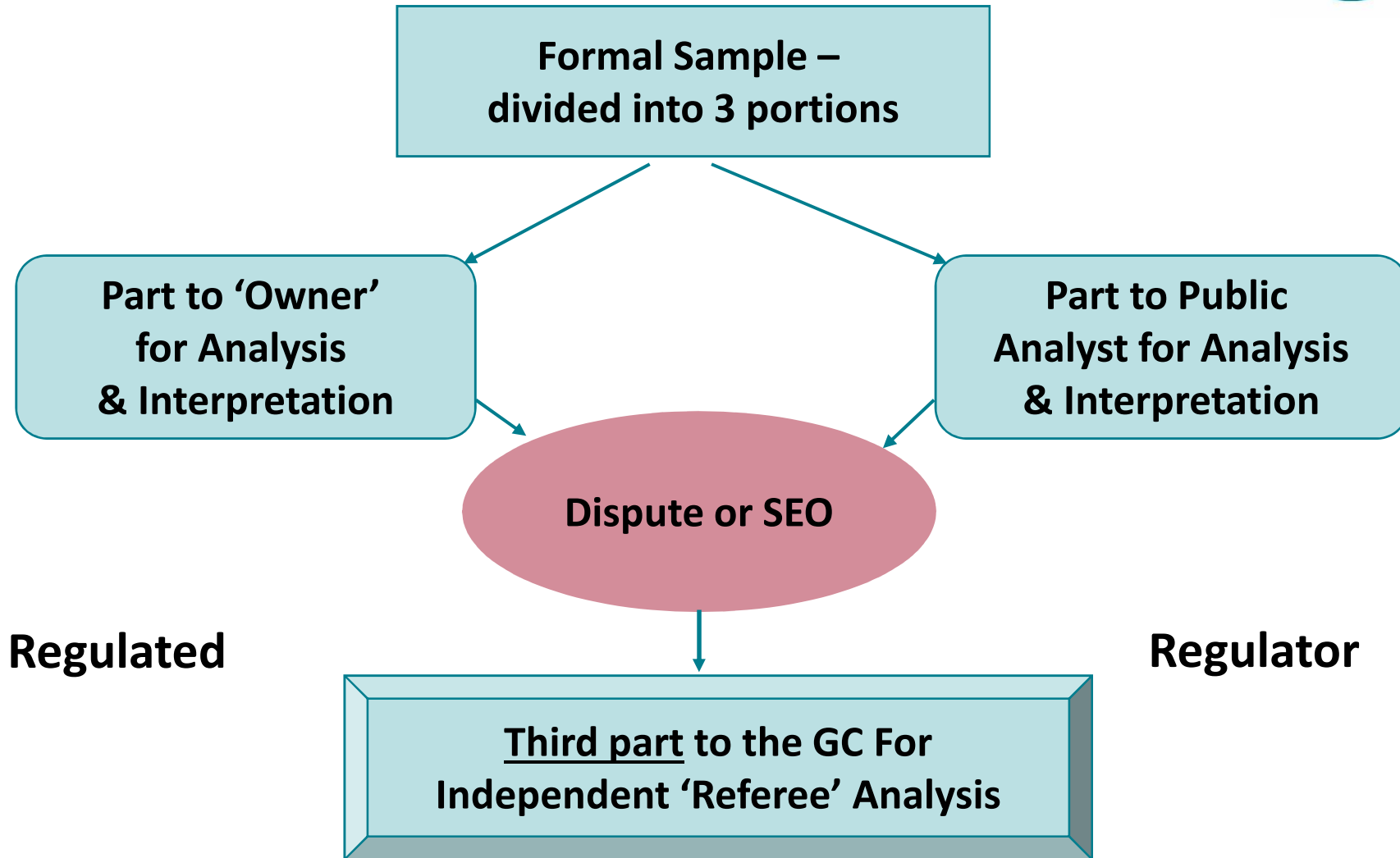
Aim of presentation

- To update stakeholders about referee case outcomes since the conference of 24th November 2014
- Knowledge transfer, transparency & governance
 - Introduction
 - Cases
 - Conclusions

Public duty



- On privatisation in 1996, LGC entered into an agreement with the Secretary of State for Trade and Industry which underpinned the continuity of the broader public functions by defining the ‘Government Chemist Function’:
 - **Government Chemist Statutory Function**
 - **Government Chemist Advisory Function**



Referee Analysis



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<p>UK Government Chemist origins</p> <p>The Laboratory of the Government Chemist was originally founded in 1842 with the remit to detect adulteration of tobacco on behalf of HM Customs & Excise.</p> <p>It continued to develop after this time to become established, for nearly half the 20th century, as a free-standing central department with a broad responsibility for the investigation and analysis of a wide range of samples and problems on behalf of other government departments and authorities.</p>	<p>Privatisation</p> <p>On privatisation in 1998, LGC entered into an agreement with the Secretary of State for Trade and Industry which underpinned the continuity of the broader public functions by defining the 'Government Chemist Function':</p> <p>Government Chemist Statutory Function</p> <p>'... as an independent and impartial referee analyst, authorised analyst and analyst by reference to certain legislation ...' [see successive annual statements of statutory scope published on http://www.gov.uk/government/chemists]</p> <p>Government Chemist Advisory Function</p> <p>'... as a source of advice for HM Government and the wider analytical community on the analytical chemistry implications on matters of policy and of standards and of regulations and the undertaking of necessary work and research to maintain the effectiveness of such advice'.</p> <p>Referee analysis takes place under both these headings.</p>
<p>Referee analyst - overview</p> <ul style="list-style-type: none"> No legal definition of the referee analyst function. Independent expert analysis and/or interpretation to help avoid or resolve disputes. Food Safety Act 1990 and the Agriculture Act 1970. Food Safety (Sampling and Qualifications) (...) Regulations 2013 (separately in England, Scotland, Wales and Northern Ireland). Formal test samples are divided into three parts by an authorised officer. 	<p>Statutory Referee Analysis - Process</p> <pre> graph TD A[Formal sample - divided into 3 portions] --> B[Part to 'house' for analysis & interpretation] A --> C[Part to Public Analyst for analysis & interpretation] B --> D((Dispute or SEO)) C --> D D --> E[Third part to the GC for independent 'referee' analysis] </pre>
<p>Conditions for statutory referral</p> <ul style="list-style-type: none"> Intention or commencement of proceedings for an offence. Prosecution intends to adduce analytical evidence. Referral can be by the local authority authorised sampling officer, the prosecutor or the court. Defendant may also, subject to agreement to deliver some or all of the Government Chemist's costs, request referral. Other legislation describes the process as 'secondary analysis' by the Government Chemist for example: <ul style="list-style-type: none"> Materials and articles in contact with food Natural mineral water, spring water and bottled drinking water 	<p>In practice (to safeguard public funds)</p> <ul style="list-style-type: none"> Evidence of a dispute is required. Administrative fee levied (a fraction of the true costs). But if statutory conditions are met a referee request cannot be refused. Financial hurdle if no evidence of dispute. Retained portion is the only sample accepted. <p>SEO - Supplementary Expert Opinion (can be obtained at any competent laboratory)</p> <p>Pursuant to Article 11(5) of Regulation 609/2004 on official controls The competent authorities shall establish adequate procedures in order to guarantee the right of feed and food business operators whose products are subject to sampling and analysis to apply for a supplementary expert opinion, without prejudice to the obligation of competent authorities to take prompt action in case of emergency'</p> <p>Advice</p> <ul style="list-style-type: none"> Recent years have seen an increased recourse to the Government Chemist as an advisor to both central and local government. <p>Agriculture Act</p> <ul style="list-style-type: none"> Similar provisions as to food but more prescription as to analytical methods and scope of analysis. <p>For further information including instructions on submission of a referee sample see www.gov.uk/government/chemists.</p>

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www.gov.uk/government/chemists



Typical steps in referee case

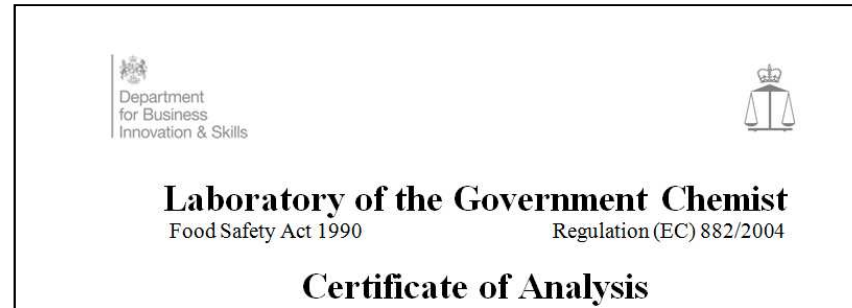
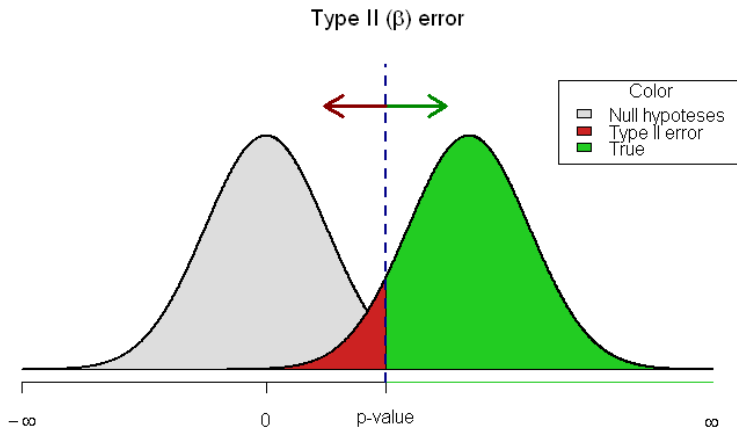


- ✓ Decision to accept
- ✓ Funding
- ✓ Schedule work
- ✓ Check legislation
- ✓ Method identified

- ✓ Method investigated ...
- ✓ Replicates 3 x 3
- ✓ CRMs, RMs spikes
- ✓ Witnessed
- ✓ > 1 technique if possible

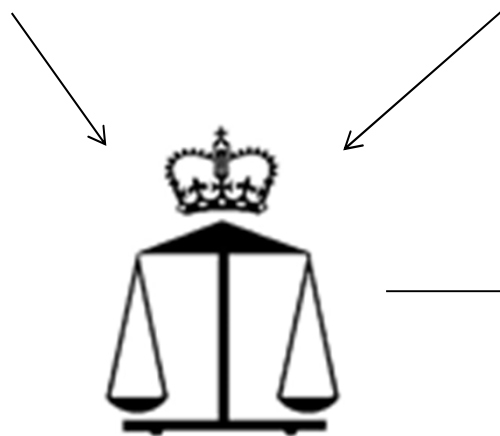
- ✓ Transcriptions checked
- ✓ Results reviewed
- ✓ New analytical runs if required

Interpretation and reporting



✓ **Statisticians review dataset**

- ✓ **Certificate drafted**
- ✓ **Reviewed**
- ✓ **Data independently checked**



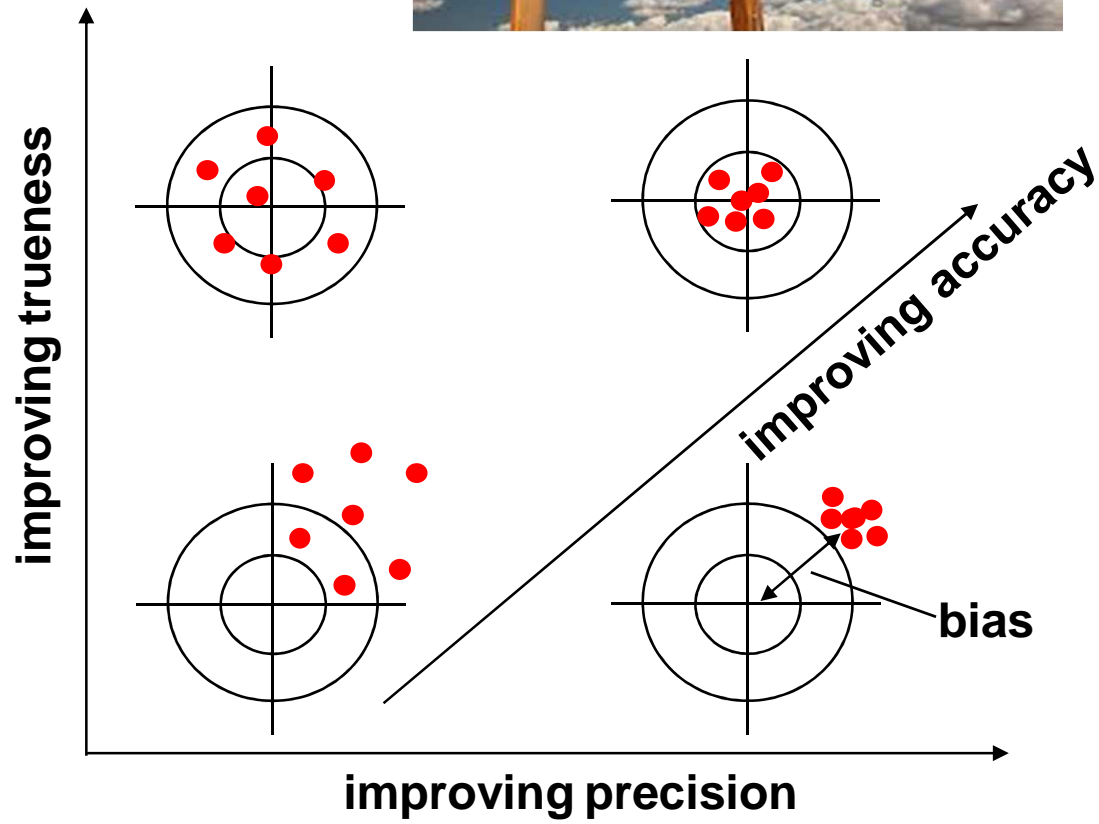
Report issued to all parties

Casework relative resource



For Public Analyst or trade laboratories such scale seldom justified

Why?





<http://loc.gov/pictures/resource/cph.3a39174/>
Robert H. Jackson by Harris & Ewing
Reproduction Number: LC-USZ62-38828 (b&w film copy neg.)
Repository: Library of Congress Prints and Photographs
Division Washington, D.C. 20540 USA



Robert H. Jackson (1892 – 1954)

Justice of the United States
Supreme Court (1941–1954)

Brown v. Allen (1953)

*“ ... we are not final because
we are infallible, but we are
infallible only because
we are final...”*

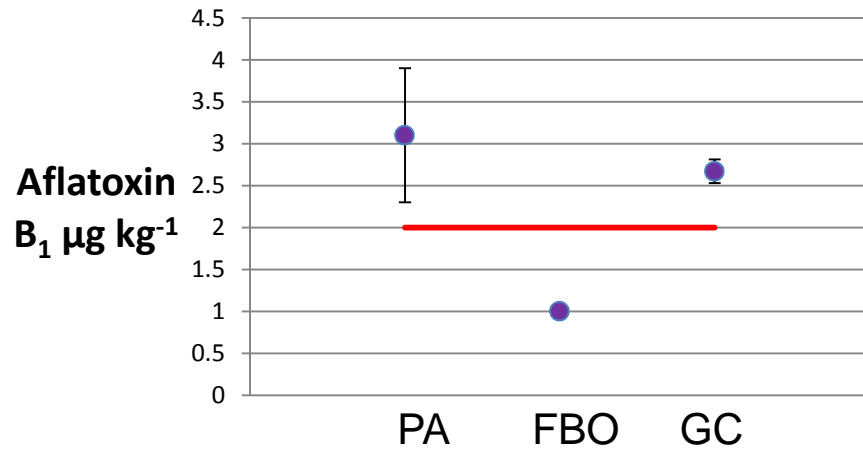
Greenhouse, L., 2004. " Because We Are Final"
Judicial Review Two Hundred Years after
Marbury. *Proceedings of the American Philosophical
Society*, 148(1), pp.38-52.



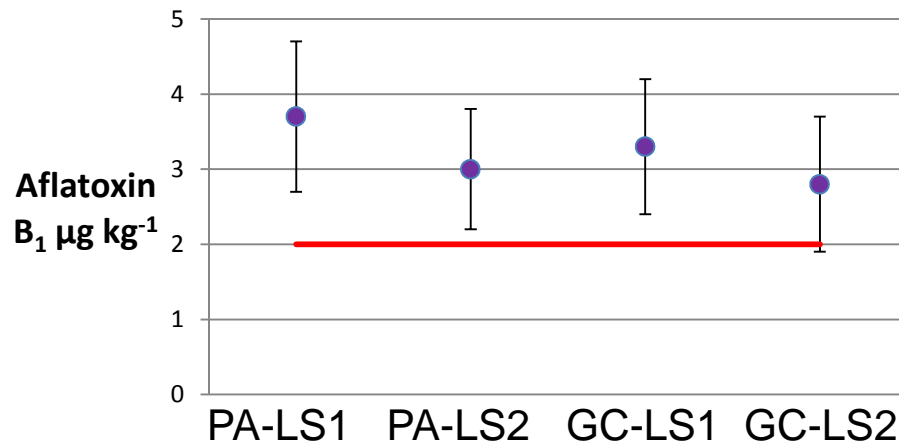
Aflatoxins in food, limits for B₁ and total ...



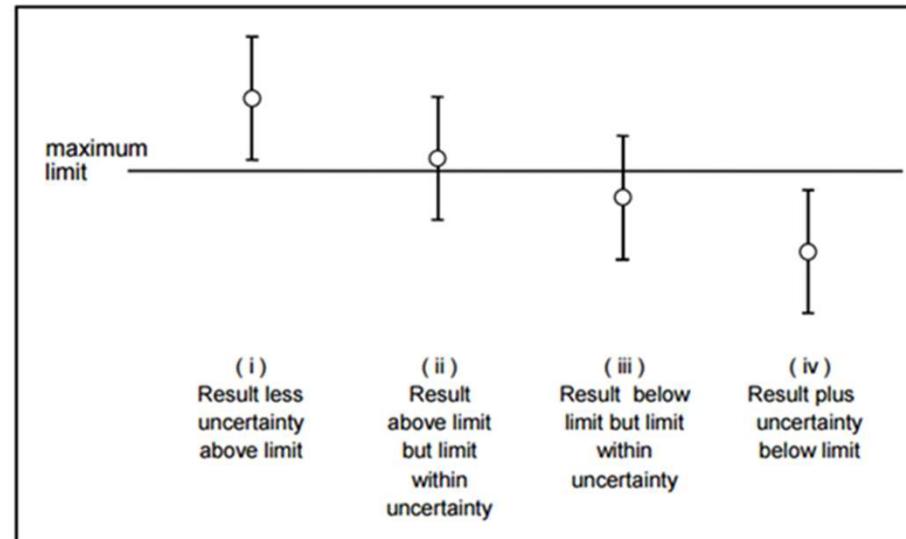
Case 1417-8 Aflatoxins in peanuts



Case 1417-18 Crunchy coconut peanuts SEO



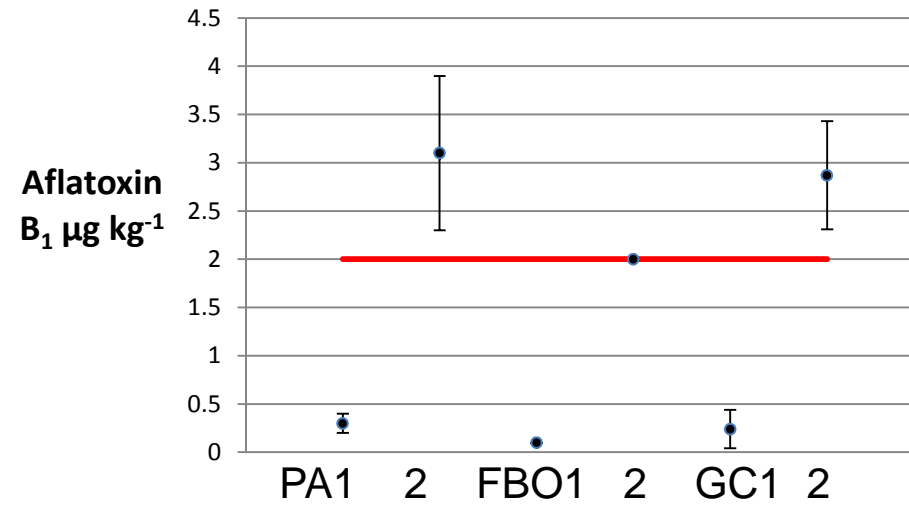
Data for total aflatoxins and LC-MS/MS confirmation not shown



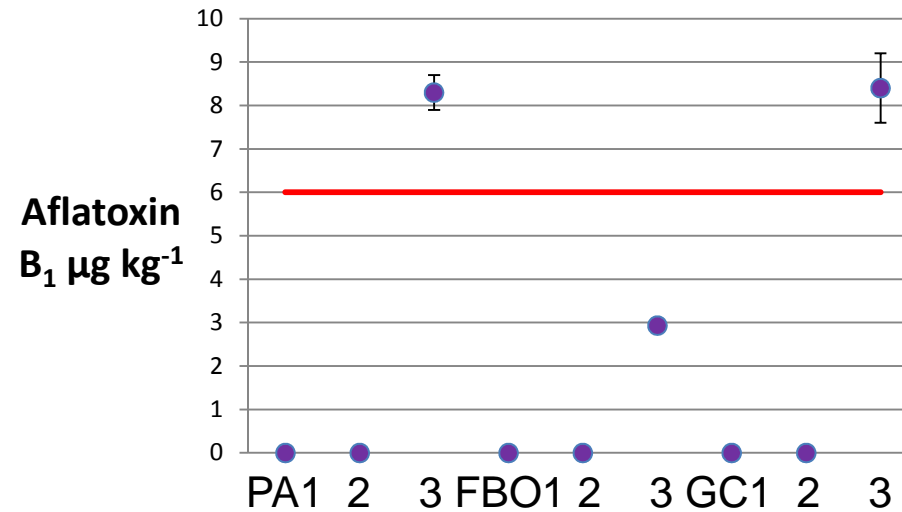
Criminal burden of proof but in multipart samples if one fails the consignment fails



Case 1417-22 Groundnut kernels



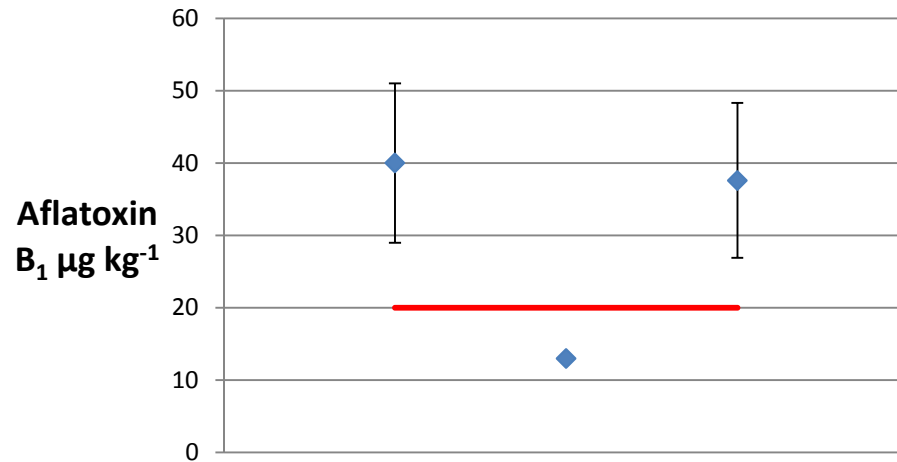
Case 1417-25 Figs



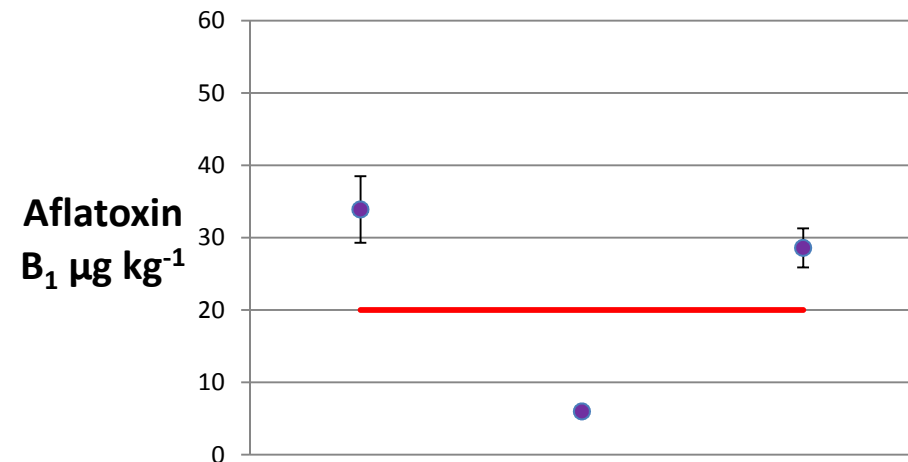


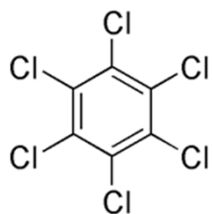
Aflatoxin B₁ in animal feed including living freely in the wild - limit 0.02 mg kg⁻¹ ... illustrated as μg kg⁻¹

Case 9 Aflatoxins in birdfeed

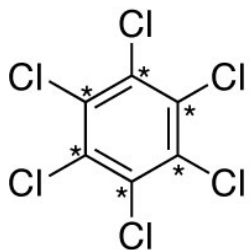


Case 32 birdfeed





**PA, FBO
multi-residue
methods $\mu \pm 50\%$**

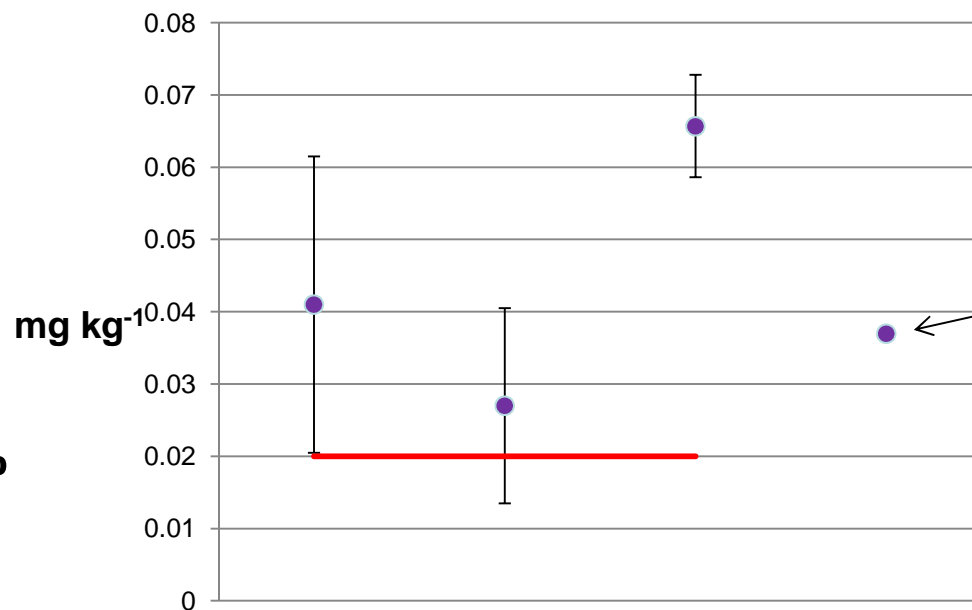


$^{13}\text{C}_6$ 99% IS

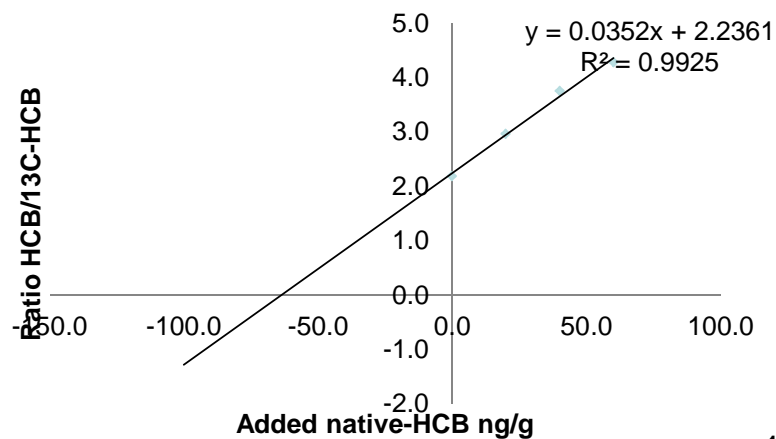
**Acetonitrile extraction
GC-MS
(Richard Brown)**

Cajka, *et al.*, Streamlining sample preparation and gas chromatography–tandem mass spectrometry analysis of multiple pesticide residues in tea, *Analytica Chimica Acta*, 743, 51-60

Case 1417-16 HCB in tea

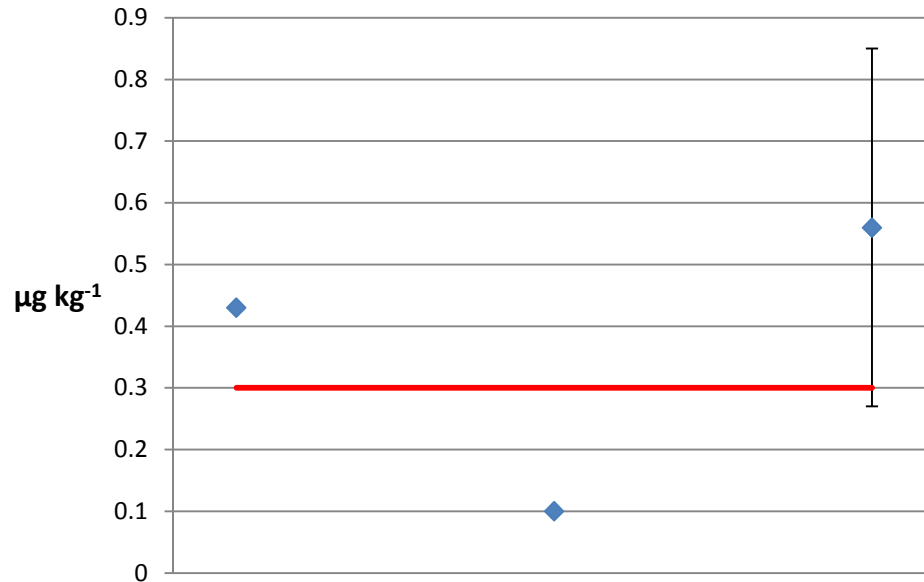


**Standard Addn. Sample A from ratio
HCB/ ^{13}C -HCB**



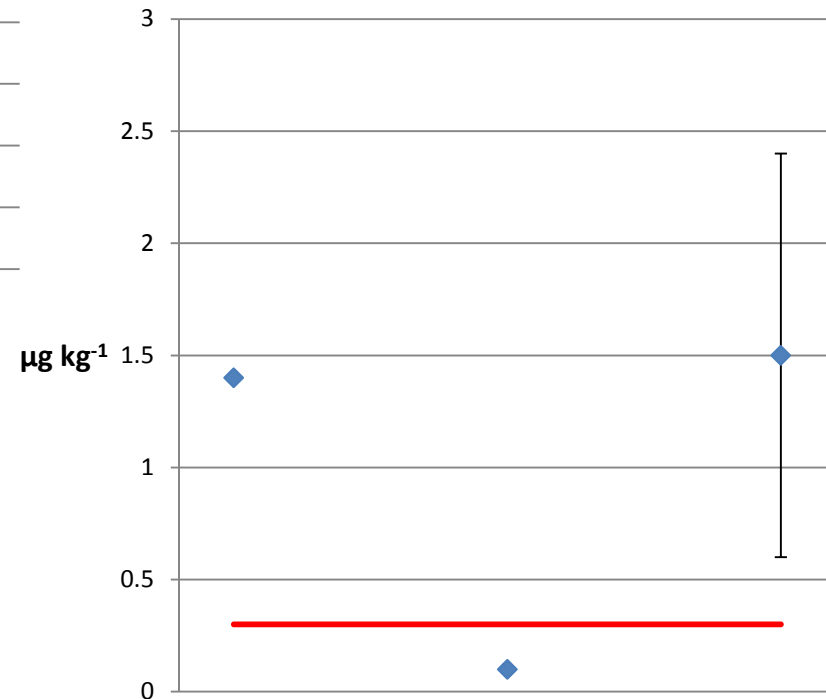


Case 1417-13 Chloramphenicol in prawns sample 1

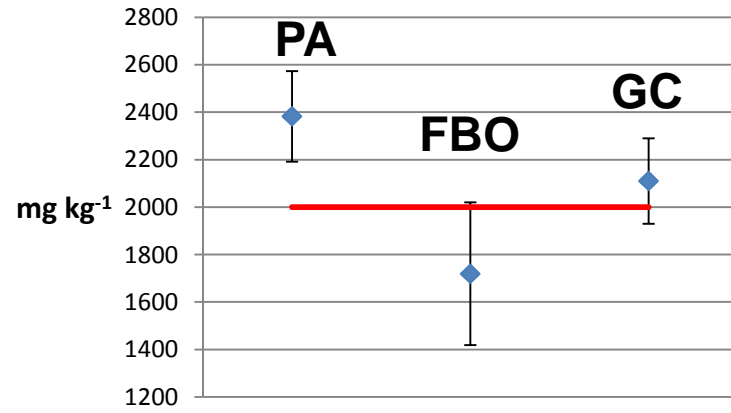


Sample thawed, drained & homogenised
+ chloramphenicol-D5 as IS, extraction &
LC-MS/MS.

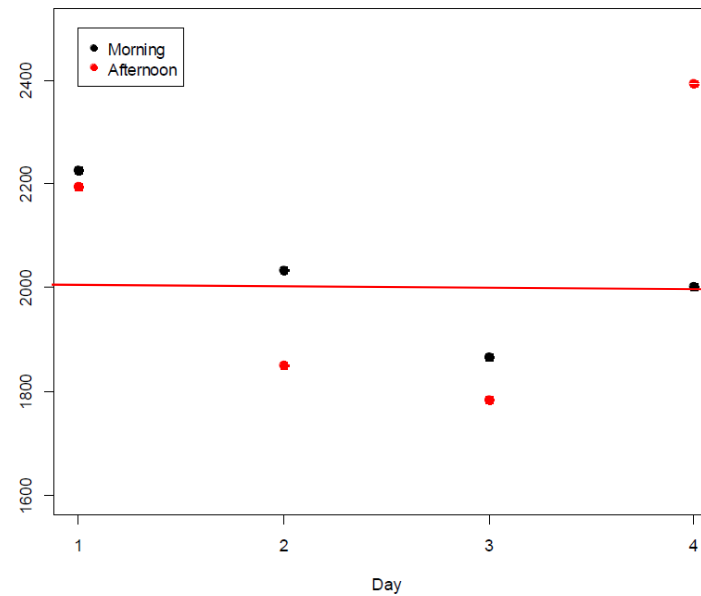
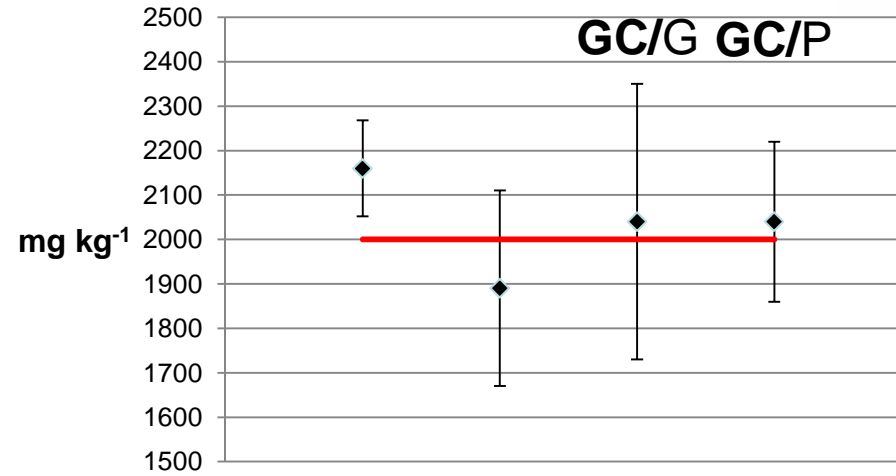
Case 1417-13 Chloramphenicol in prawns sample 2



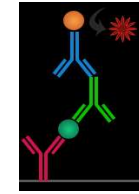
Case 1417-14 SO₂ in dried apricots



Case 1417-14 SO₂ in dried apricots



Case 1417- 24 Speciation



**Sold as Goat
Was in fact Sheep**



**Sold as Sheep
Was in fact Beef**



**Sold as lamb
Was in fact mixture of
Sheep and chicken**

**Completely confirmed
Public Analyst's
Findings –**

Guilty verdict

Analytically right and analytically final ...



Aflatoxins

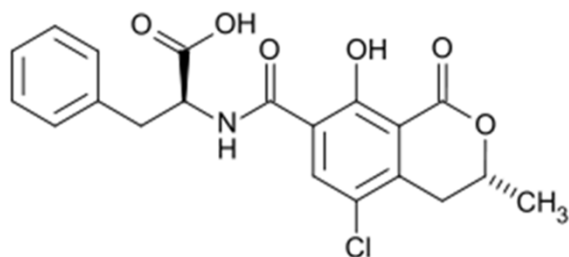
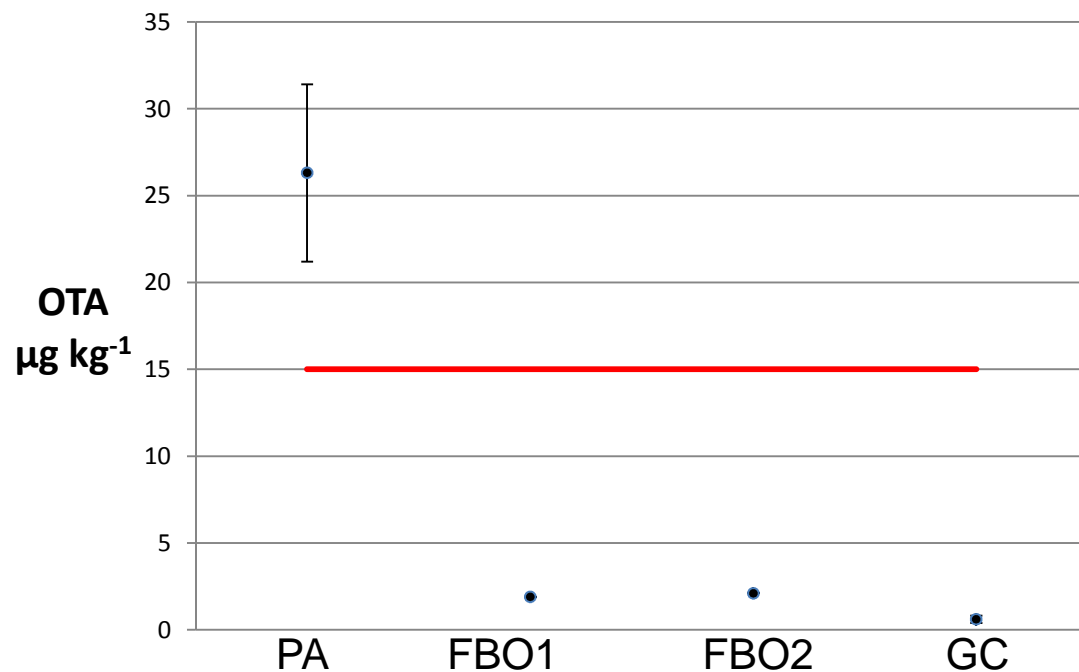
- Commission Regulation (EC) No 401/2006 *laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs*
- Up to 100 incremental samples combined



Pesticides, Veterinary Residues, Speciation ...

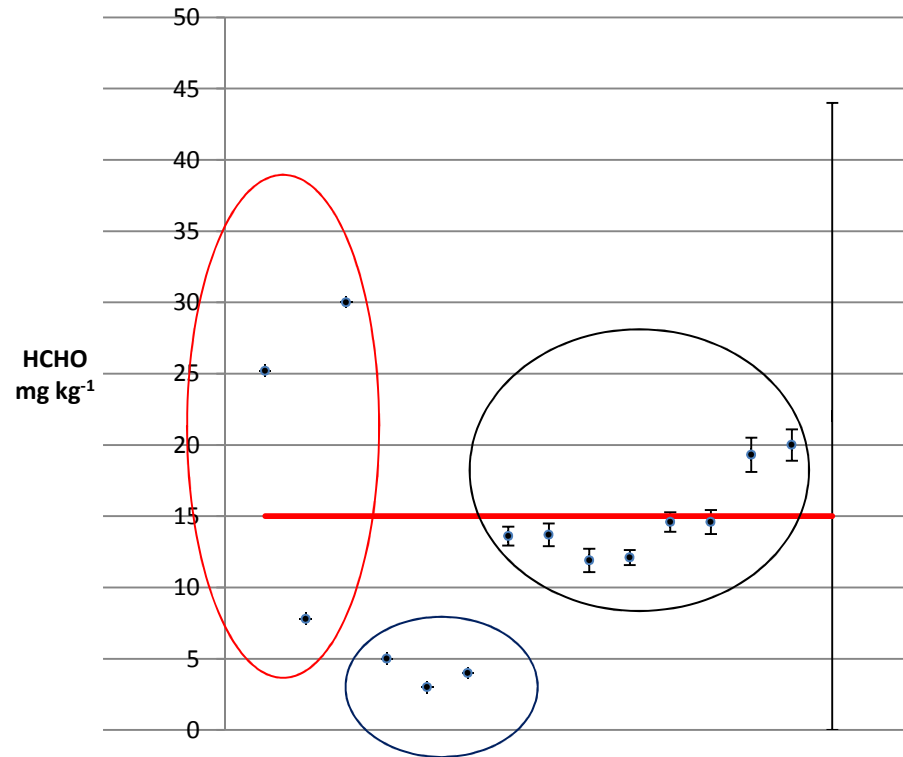
Extent of replication, informed interpretation ...

Case 1417-31 OTA in ground black pepper



- ✓ Incrementally sampled
- Not slurried
- ❖ No advice on sample homogenisation and division

Case 15 FCM



$$\frac{u^2(C)}{C^2} = \frac{u^2(F_s)}{F_s^2} + \frac{u^2(M_s)}{M_s^2} + \frac{u^2(A)}{A^2}$$



$$C = 6 \frac{F_s M_s}{A}$$

$C = [\text{HCHO}] \text{ mg kg}^{-1}$ equivalent to mg per 6 dm²

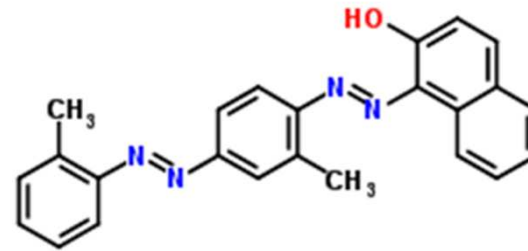
$F_s =$ milligrams formaldehyde per kilogram simulant

$M_s =$ fill volume in kg

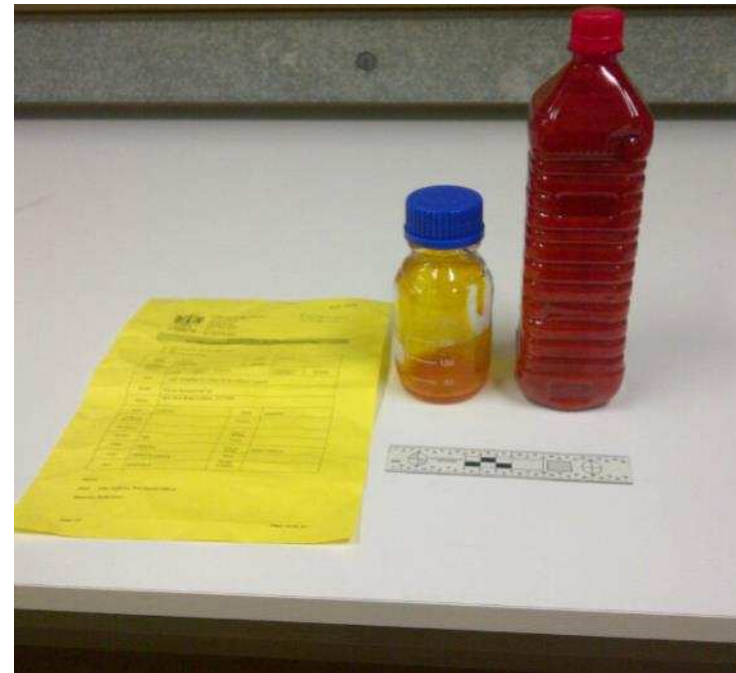
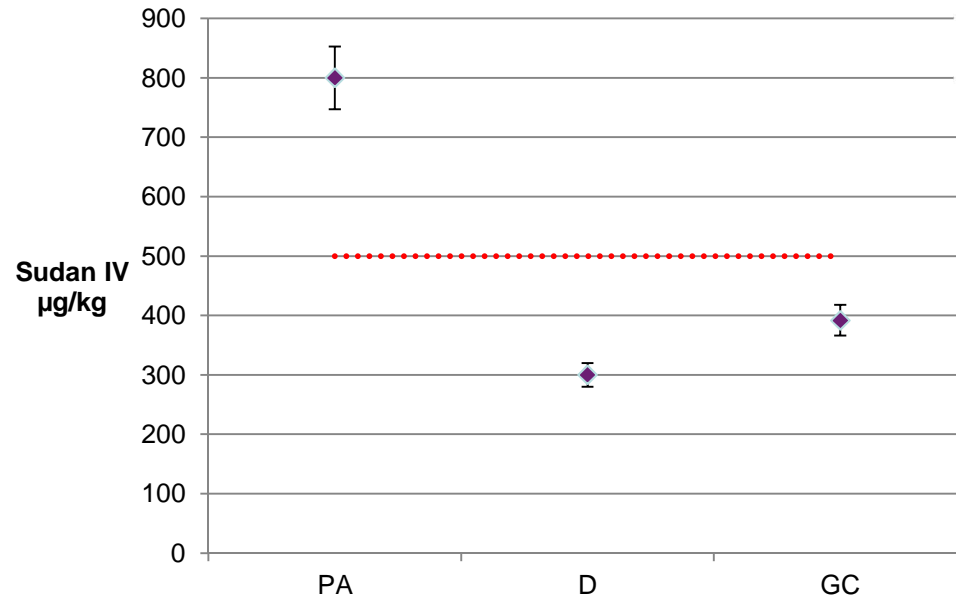
$A =$ Surface area in dm²

mean A and M_s for each individual bowl and their respective standard errors as uncertainty components.

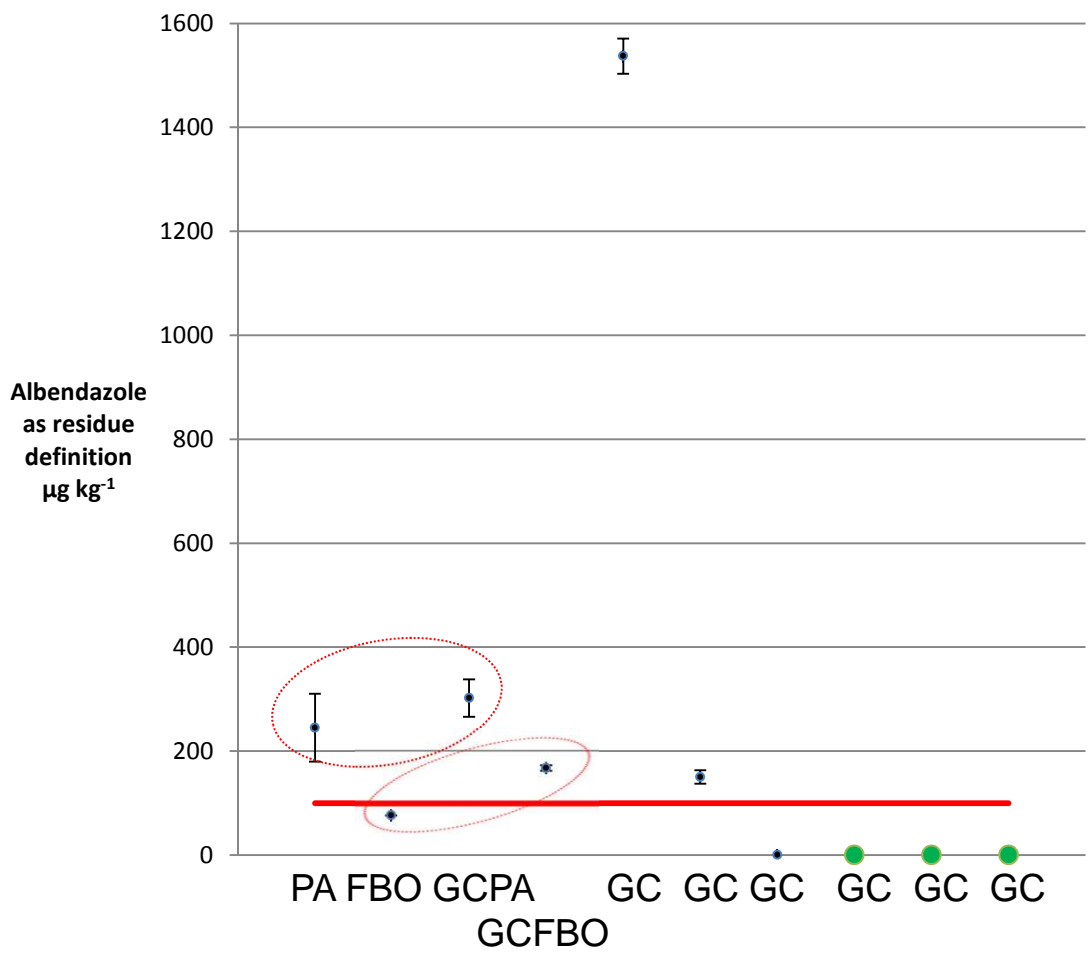
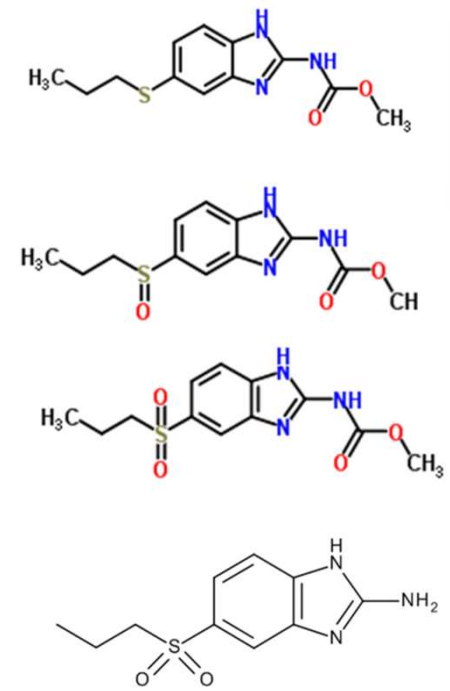
Case 1417-26 Palm Oil



Palm Oil Sudan IV



Case 1417-23 Albendazole



Resolution of a Disputed Albendazole Result in the UK Official Control System – Time for More Guidance?

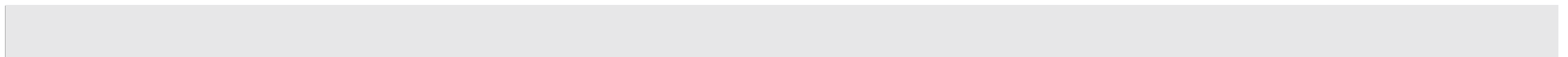
Michael Walker^a, Kirstin Gray^a, Christopher Hopley^a, Christopher Mussell^a, Louise Clifford^a, Jayanie Meinerikandathevan^a, Leonardo Firpo^a, Joanna Topping^a and Daniel Santacruz^b.
^a LGC Queens Road Teddington Middlesex TW11 0LY, UK
^b London Port Health Authority, Stanford-Le-Hope, Essex, SS17 9LA, UK
 Correspondence: michael.walker@gcgroup.com

UK Government Chemist Funded by the UK Department of Business, Innovation and Skills, the Government Chemist	Referee Case: Albendazole in import of tinned Corned Beef Albendazole is an anthelmintic drug used in ruminants.
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Speciation

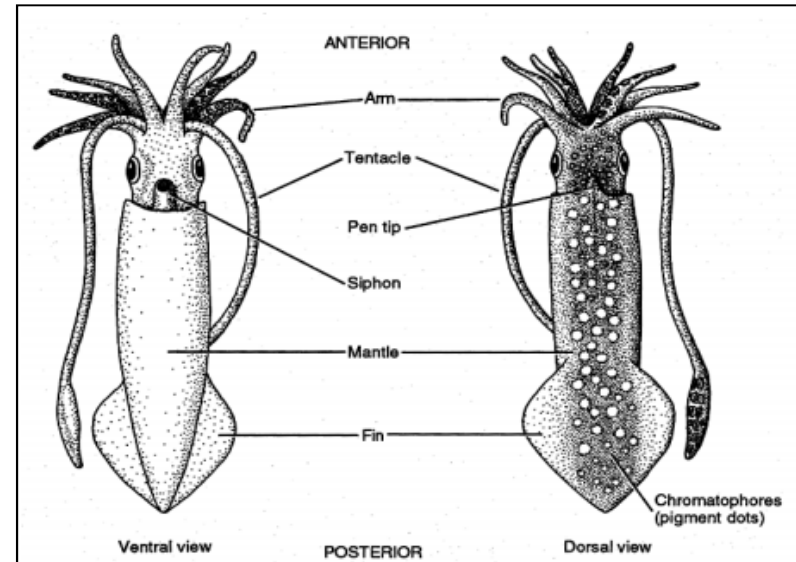
Squid



Case 1417-5 Squid



back label -- ingredients stated “squid” and “Produced in New Zealand and packed in the UK from **arrow squid** caught in the South West Pacific Ocean for ...[address of retailer]”





Dispute

- Arrow squid is the commercial designation for squid of the species *Nototodarus gouldi* and *Nototodarus sloani*
- Public Analyst certified that DNA extracted from the sample was consistent with that of *Illex argentinus* or the 'Argentine short fin squid'
- Laboratory acting for the FBO reported that their portion contained DNA of *Nototodarus gouldi* and *Nototodarus sloani* consistent with the label information.

Squid



Suborder	Family	Genera	UK official sales designation and species ^{§ 12}
<p>Oegopsida eye open to the water, open ocean animals living at the surface down to at least 3,000m</p> <p>There are about a dozen other families of squid in the Oegopsida suborder such as Onychoteuthidae, Gonatidae, Architeuthidae and Enoplotheuthidae that do not have sales designations in the UK.</p>	Ommastrephidae	<p><i>Ommastrephidae</i>, <i>Illex</i>, <i>Todarodes</i>, <i>Nototadorus</i>, <i>Todaropsis</i>, <i>Dosidicus</i>, <i>Symplectotheuthis</i></p>	<p>Squid: <i>Ommastrephes sagittatus</i> All species of <i>Illex</i></p>
			<p>Arrow squid: <i>Nototadorus gouldi</i>, <i>Nototadorus sloani</i></p>
			<p>Pacific squid: <i>Todarodes pacificus</i></p>
			<p>Squid or Jumbo Squid or Giant Squid: <i>Dosidicus gigas</i></p>
<p>*Myopsida Eye covered by transparent membrane neritic (coastal waters) to inshore animals</p>	Loliginidae	<p><i>Loligo</i>, <i>Doryteuthis</i>, <i>Alloteuthis</i>, <i>Sepioteuthis</i></p>	<p>Squid: All species of <i>Loligo</i></p> <p>Chinese squid: <i>Uroteuthis chinensis</i>, also known as <i>Loligo chinensis</i></p>

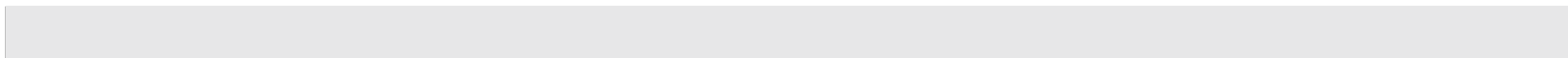
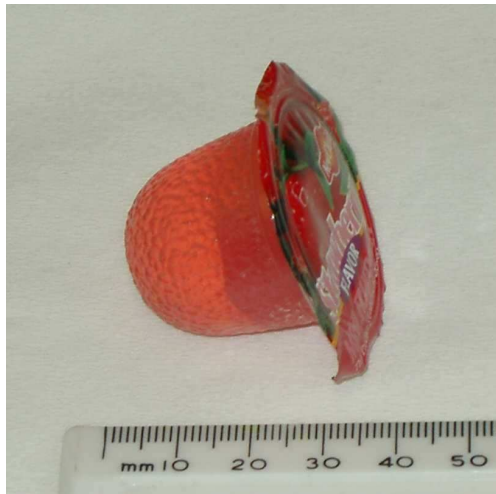




Approach

- Sequence identified, primer designed and externally synthesised
- Extract DNA, DNA target region amplified by PCR
- Sequencing performed externally on amplified DNA
- Analysis of the sequences generated, Phylogenetic trees
- BOLD gave both *Illex* and *Nototodarus* as most probable species, > 99% similarity with target sequence
- NCBI, database gave both *Illex* and *Nototodarus* species shared joint top most probable species identity, 89 % - 94 % sequence similarity with the referee sample sequence.
- Both Public Analyst and FBO laboratories were justified in their differing reported findings
- Taxonomic difficulties in the cephalopoda are well recognised
- Only a limited number of relevant individual specimens of *Illex* and *Nototodarus* that have been sequenced, as reported in a small number of peer reviewed publications

Jelly mini cups - alleged choking hazard





Definition

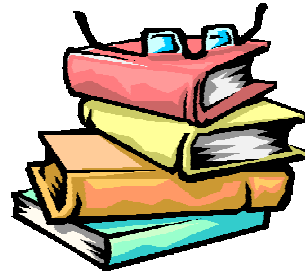
Part E of Annex II of Regulation 1333/2008

“The substances listed under numbers E 400, E 401, E 402, E 403, E 404, E 406, E 407, 407a, E 410, E 412, E 413, E 414, E 415, E 417, E 418, E 425 and E 440 may not be used in jelly mini-cups, defined, for the purpose of this Regulation, as **jelly confectionery of a firm consistence, contained in semi rigid mini-cups or mini-capsules, intended to be ingested in a single bite by exerting pressure on the mini-cups or mini-capsule to project the confectionery into the mouth;** E 410, E 412, E 415 E 417 may not be used to produce dehydrated foods intended to rehydrate on ingestion. E425 may not be used in jelly confectionery.”

Food Anal. Methods (2012) 5:54–61
DOI 10.1007/s12161-011-9223-3

Analytical Strategy for the Evaluation of a Specific Food Choking Risk, a Case Study on Jelly Mini-Cups

Michael J. Walker • Peter Colwell • Derek Craston •
Ian P. Axford • Jack Crane



Jelly confectionery? ✓

Firm consistence?

Intact and unchanged in saliva for > 2 mins, even sample
'very soft and pliable with little structural strength' ✓

Contained in semi rigid mini-cups or mini-capsules? ✓

Ingested in a single bite ✓

Remitted to FSA for examination of a formal sample by a third Public Analyst

Will publish updated advice

Cumin



Specification	Suggested limit
<i>ASTA cleanliness specifications</i>	
Whole insects, dead (No.)	4
Mammalian excreta (mg/lb)	3
Other excreta (mg/lb)	5
Mould (% by weight)	1
Insect-defiled/infested (% by weight)	1
Extraneous foreign matter (% by weight)	0.5
Ash (% max)	9.5
Acid-insoluble ash (% max)	1.5
<i>FDA DAL</i>	
Volatile oil (% min)	2.5
Moisture (% max)	9.0
Ash (% max)	8.0
Acid-insoluble ash (% max)	1.0
Average bulk index (mg/100g)	240
<i>USFDA DAL</i>	
Sand and grit (AOAC 975.48)	Average of 9.5% or more ash and/ or 1.5% or more acid-insoluble ash

Adulterants

- Caraway
- Sand, grit & debris
- Milled foliage, straw, wheat & rice husks
- Starches
- Olive stones
- Essential oil can be adulterated with synthetic cumin aldehyde
- Peanut & treenut by-product?
- ?????



Cumin recalls

- Oct 2014 Canadian Food Inspection Agency random tests for allergens revealed undeclared peanut & almond protein in products containing cumin
- Widespread recalls of cumin products in N America (42 to 19 Feb 2015)
 - one for **peanut and almond** starting in Autumn 2014, and a second larger one for **peanut only**, starting in December 2014
 - Salsas, spices inc paprika & mixes, seasonings → meat products, hummus,
 - None of the N American recalled products distributed in UK

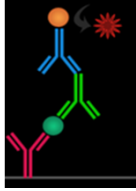
Sayers *et al.*, 2016, How Much of a Problem Is Peanut in Ground Cumin for Individuals with Peanut Allergy? *J Allergy Clin Immunol*, 137, AB99, February 016, Abstract 467


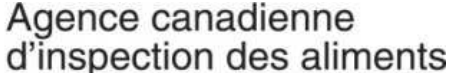
Sources – Patrick Bennett, Allergic Living, USA, <http://allergicliving.com/2015/02/14/inside-the-peanut-tainted-cumin-recalls-what-happened/>



More + UK & Europe recalls

- Jan 31 2015 FSA recalled ground cumin with almond protein, on a precautionary basis
- February 2015 FSA issued two further recalls re undeclared almond protein in fajita meal/dinner kits and seasoning mixes
- Batch of paprika was the likely source (Santa Maria)
- Denmark, Sweden & Norway issued alerts / recalls
- None of the tests have detected peanut proteins at levels that would require allergen labelling (FSA)



- One ELISA ...”Cross reaction to apricot stone is > 100 %”
- Several papers PCR cross reactivity ...
- Barts Ingredients Company Ltd, the manufacturer of the UK cumin recalled claimed (early March 2015) that mahleb, a little known nut, was possibly the origin of the ELISA almond positives
- 30 April 2015  Canadian Food Inspection Agency  Agence canadienne d'inspection des aliments
 - CFIA rescinded ~ 25 product recalls ...
 - “... new evidence regarding the cross-reactivity of mahaleb, a spice obtained from a specific species (*Prunus mahaleb*) of cherry seeds, with the almond allergen test kit. It is highly likely that the positive sample results for the ground cumin and cumin-containing products were due to mahaleb contamination and not almond”

Experimental plan

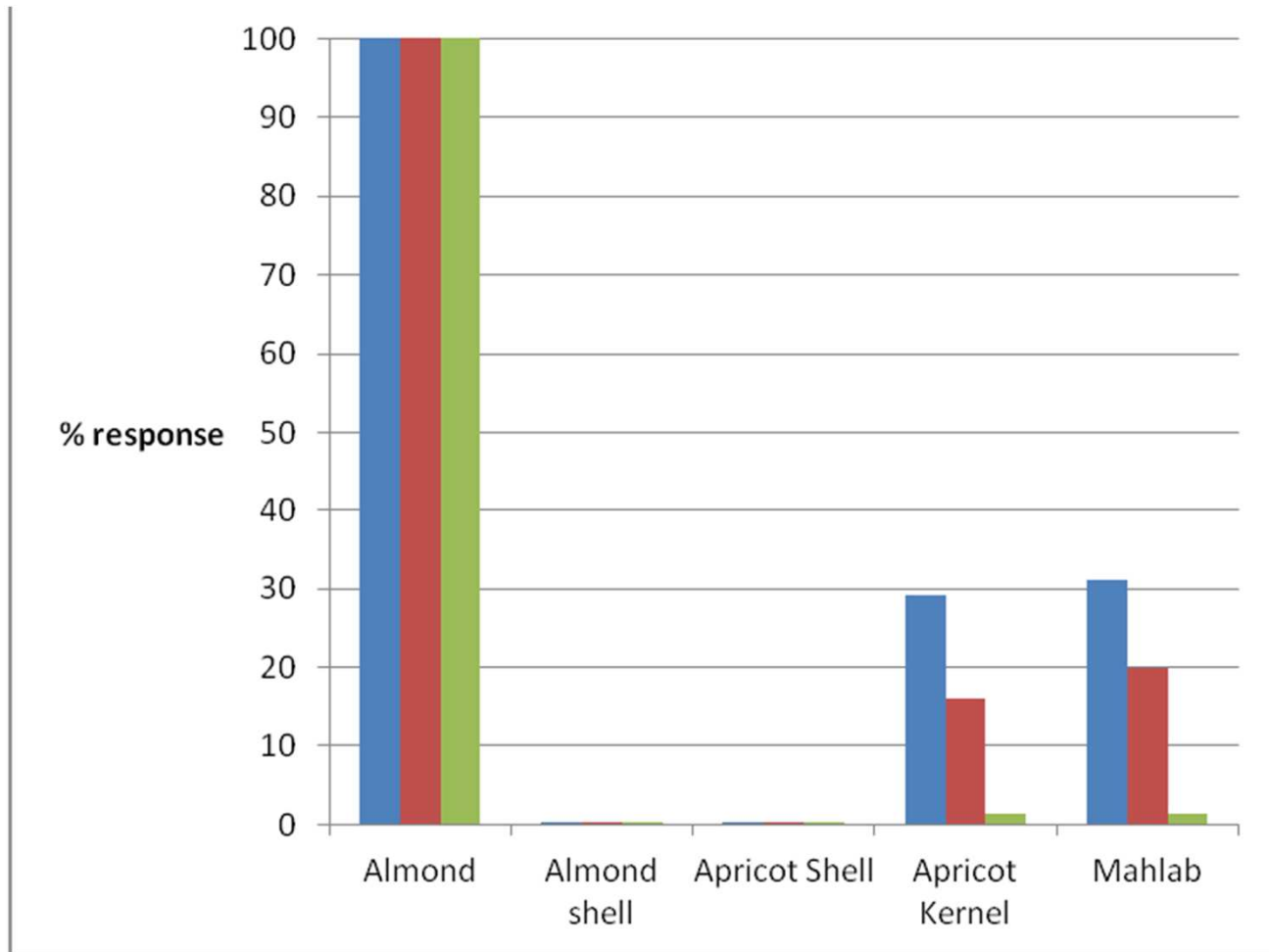


Sample appearance - ground cumin, 21.5 g

- Multiple ELISAs
- Searches of bioinformatic databases for DNA sequences
- A real-time PCR DNA assay developed for this investigation
- Investigation of control material by liquid chromatography quadrupole time of flight mass spectrometry, LC-ToFMS and
- Protein analysis by triple quadrupole liquid chromatography - tandem mass spectrometry (LC-MS/MS) of enzymatically digested aliquots of the sample



ELISA cross reactivity



Replicates n =3, on one day, including milled apricot and almond shells

ELISA Results for cumin referee sample



ELISA Platform:	A	B	C
Presumptive Prunus protein mean result mg kg ⁻¹ (n =3) expressed as almond	11.1	1.4	3.3
<i>U</i>	1.1	0.1	0.4
Prunus protein <i>not less than</i> mg kg ⁻¹ expressed as almond	10.0	1.3	2.9
LoQ	0.4	0.5	2.5

Overall findings in cumin referee sample



Technique	Almond kernel	Mahaleb kernel
Commercial ELISAs (n = 3 x 3 = 12)	<i>A Prunus species protein was detected above the limit of quantification of three assay platforms with statistical significance</i>	
Real time PCR in-house Assay (n =12 PCRs)	No assay available	Mahaleb DNA Detected
LC-MS/MS	No peptides uniquely characteristic of almond were detected	Of the 6 peptides found in control mahaleb material but not in almond 2 were detected and 4 were not detected.



Cumin / Paprika – almond / mahaleb

- FSA asked us to look at Paprika
 - Real world sample preferable
 - Properly sampled
 - Hence retained portion obtained
 - Consignment had not entered UK food chain
 - Similar experimental plan to cumin ...



Paprika ELISA results

ELISA Platform:	A	B
Presumptive Prunus protein mean result mg kg ⁻¹ (n =3) expressed as almond	590	390
<i>U</i> *	± 110	Not available
Prunus protein <i>not less than</i> mg kg ⁻¹ expressed as almond	470 [§]	Not available

U: Expanded measurement uncertainty expressed as a 95 % confidence interval; Between-run variation and other associated sources of uncertainty have not been included.

§ Rounded outwards to 2 significant figures

Summary Paprika Results



Technique	Almond kernel	Mahaleb kernel
Commercial ELISAs (n = 2)	Prunus species protein detected About 400 – 700 mg kg ⁻¹	
Real time PCR in-house mahaleb-specific assay	No test available	Mahaleb DNA Not Detected
Real time screening PCR Prunus assay	Almond DNA Detected	Mahaleb DNA Not Detected
LC-MS/MS	Two peptides uniquely characteristic of almond were detected	No peptides uniquely characteristic of mahaleb were detected

<https://www.gov.uk/government/news/paprika-referee-sample-further-testing-identifies-almond-present>



12:40 **Lunch**

14:00 **Managing food allergies in the real world**

Paul Turner, Imperial College London

14:30 **Development of advanced PCR methods for testing the authenticity of herbs and spices**

Gavin Nixon, LGC

15:00 **Mass spectrometry – a powerful technique for identification of protein allergen contaminants**

Milena Quaglia & Chris Hopley, LGC



Conclusions (cumin / paprika)

- ELISA is a good screening technique for Prunus
- But confirmation by PCR or LC-MS/MS is required
- Spice supply chains are vulnerable
- Current issues probably adventitious rather than fraudulent
 - Cross contamination at milling is extensive
 - Our findings indicate an unknown Prunus species present
- Pose a risk for almond allergic individuals owing to the amino acid homology

Conclusions (cumin / paprika) continued



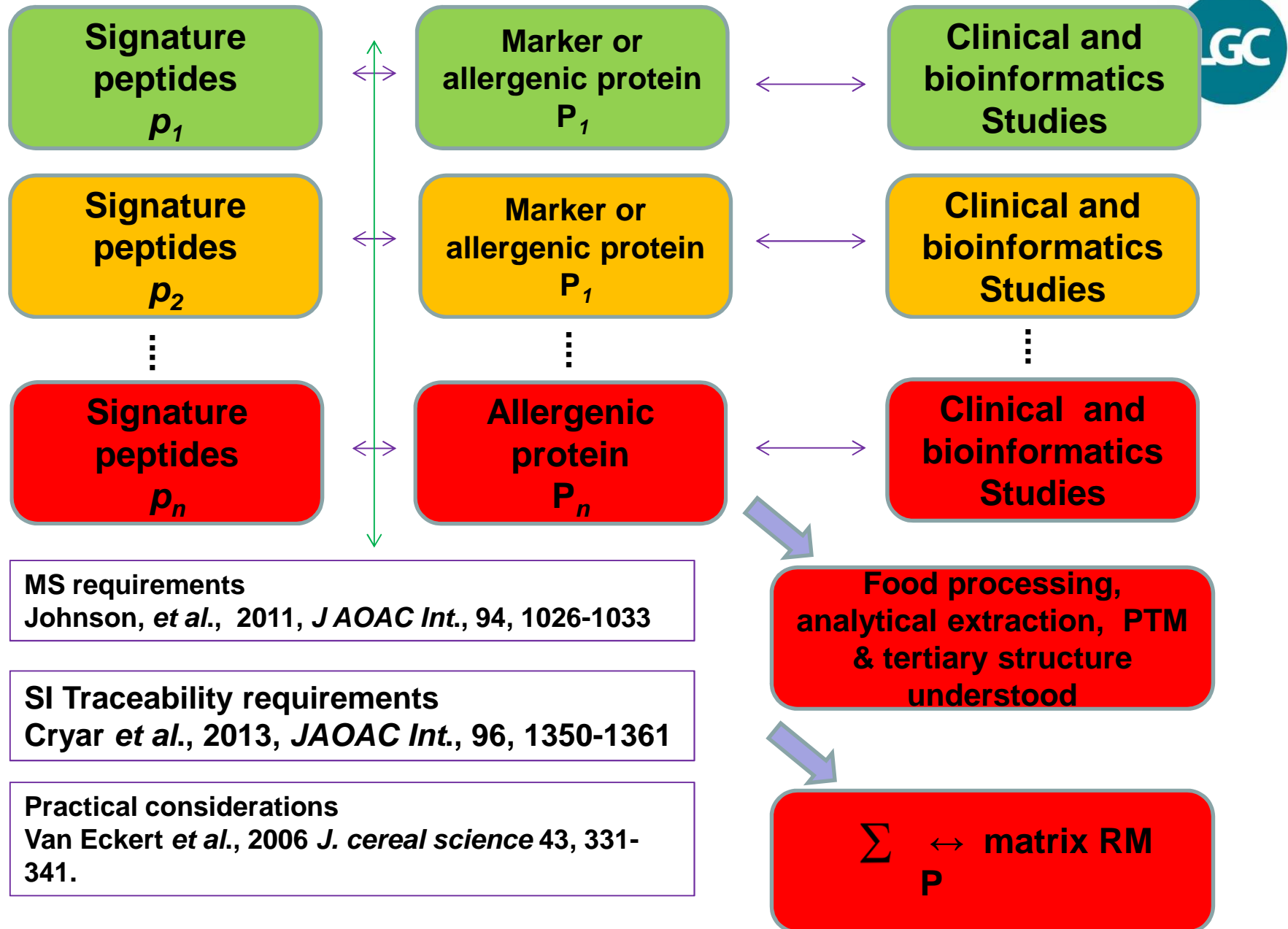
- Fortunately
 - Prunus concentrations relatively low
 - Dilution in end-use foods high
 - Almond allergy not highly prevalent
- But lack of analytical scrutiny leaves the supply chain open to a major problem either by
 - Gross cross contamination
 - Deliberate fraud
- Risk assessment hampered when specific detection and quantification remain problematic

Problems



- All allergen analytical methods flawed
 - Bioinformatics insufficient
 - No SI traceable calibrants, RMs, CRMs
 - No SI traceable reference methods

The screenshot shows the top portion of a journal article page. At the top right is the Royal Society of Chemistry logo. Below it, the journal title 'Analyst' is displayed. A dark grey bar contains the text 'CRITICAL REVIEW' on the left and 'View Article Online' and 'View Journal' on the right. Below this bar is the CrossMark logo and the text 'Cite this: DOI: 10.1039/c5an01457c'. The main title of the article is 'Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs'. Below the title are the authors: 'M. J. Walker,^a D. T. Burns,^b C. T. Elliott,^b M. H. Gowland^c and E. N. Clare Mills^d'.





Conclusions



“... last because we are right...?”

- Rigorous sampling, sample homogeneity, well characterised methods, extent of replication, informed interpretation ...

“... right because we are last...?”

- Sample homogeneity difficult or impossible, methods require investigation, boundaries of current science ...



But our aim remains to safeguard regulators, businesses & courts from unwitting errors in measurement science and, as a backstop against any possible shortcomings in sampling or analysis I hope I have shown appeal to the Government Chemist is a viable and equitable process



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