Government Chemist

Resolution of technical disputes in the UK official food control system – **Why do labs get it wrong?**

Michael Walker APHA Conference 7th November 2019

NML UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC

LGC

Question 1

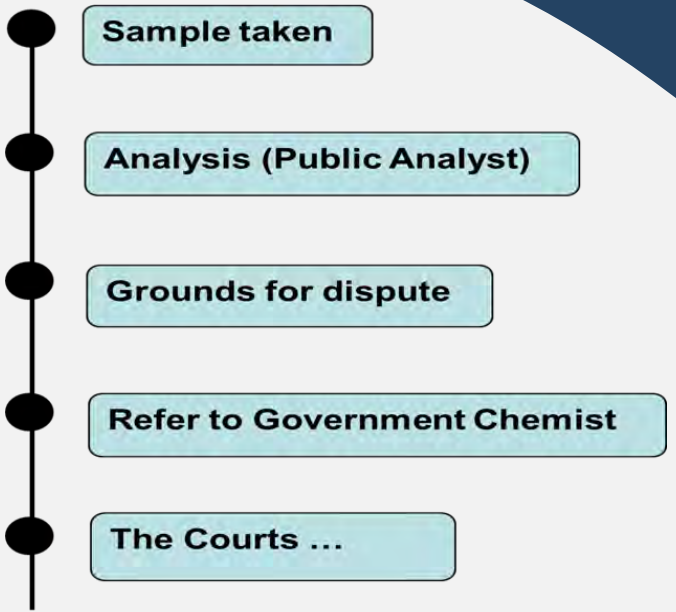
Laboratories reporting results of food analysis sometimes give the wrong results or the wrong interpretation – why?

Question 2

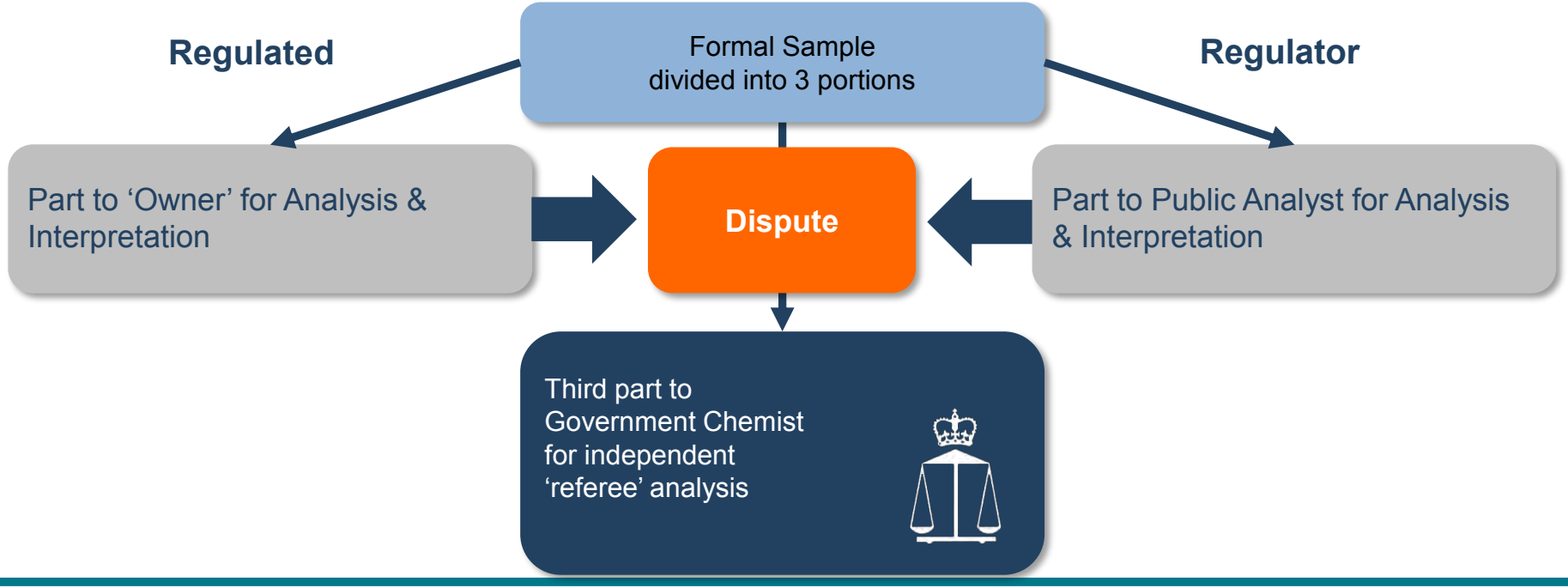
On what basis can it be said that laboratories reporting results of food analysis sometimes give the wrong results or the wrong interpretation?

Government Chemist acts …

- As an **independent referee analyst**, resolving disputes that occur in relation to certain legislation
- As an **advisor** to the public sector and the wider analytical community, where there are measurement science implications of existing and proposed legislation and regulation



Statutory referee function – typically …

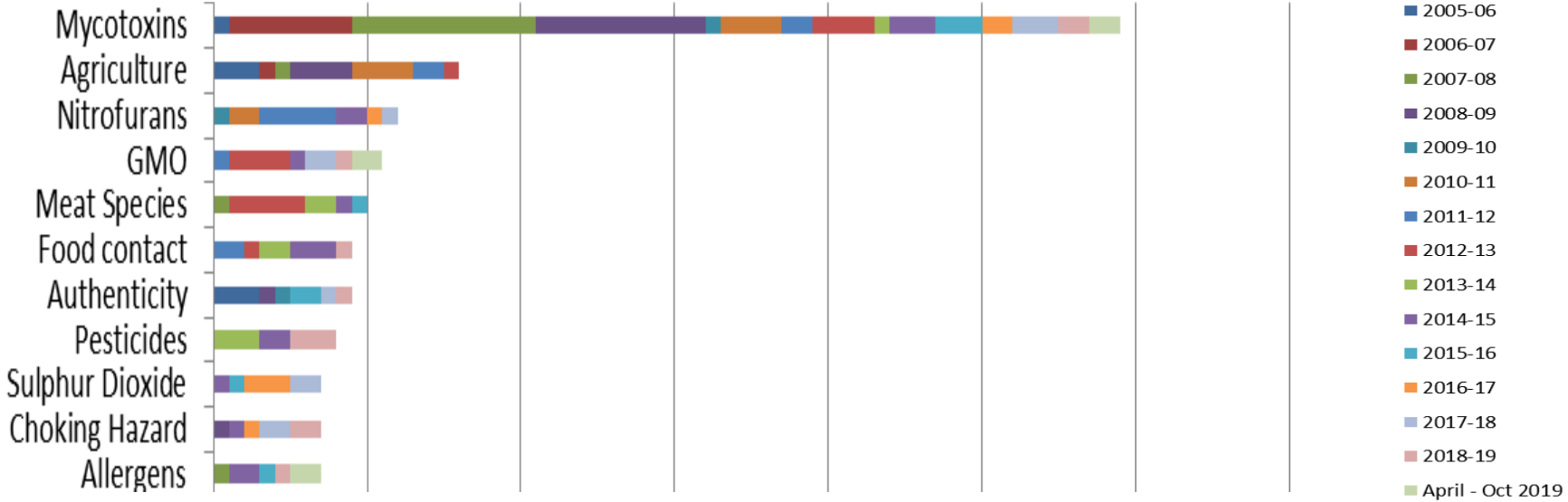


Typical steps in a referee case…

1. Accept referral?
2. Funding
3. Schedule work
4. Check legislation
5. Identify method
6. Investigate Method
7. **Replicates 3 x 3**
8. CRMs, RMs spikes
9. **Witnessed**
10. **Orthogonal** confirmation if possible

11. Transcriptions checked
12. Results reviewed
13. New analytical runs if required
14. **Statisticians** review dataset
15. Certificate drafted
16. Reviewed
17. Data independently checked
18. **Peer review**
19. Certificate issued to all parties

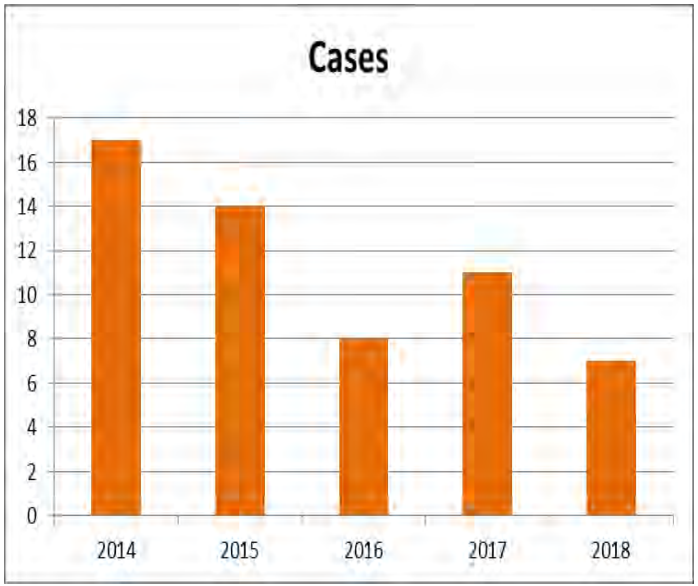
Overview of GC referee cases – cumulative by type

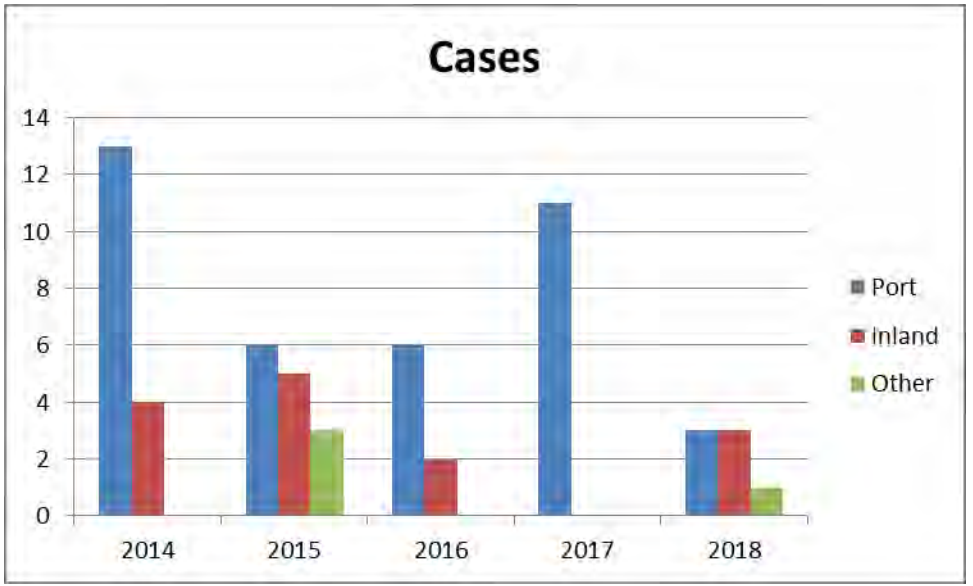


NML
NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT
HOSTED AT LGC

LGC

Cases origin





Casework relative resource

Casework relative resource

Find out more …

GOV.UK

Search

Home > Organisations > Government Chemist

Government Chemist

Submit a referee sample to the Government Chemist

Submit a supplementary expert opinion sample

Part of Department for Business, Energy & Industrial Strategy

Featured

24 July 2019 — Corporate report

Government Chemist Annual Review 2018

Referee casework, research projects, advice and impact of work carried out by the Government Chemist team

NML UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC

LGC

The Association of Public Analysts

Protecting the Public | Search | Go

Home | About Us | What We Do | News | Training | Careers | Tenders | Links | Contact Us | Members

Protecting the Public

The Association of Public Analysts (also known as the APA) is the professional body for the highly-skilled scientists who make up the front-line of the UK's public protection service. The Public Analyst works to ensure the health and safety of the public in relation to food, animal feed, water, environmental matters and consumer protection.

Headlines

APA CONFERENCE -GLASGOW 2019

This year's APA conference will be held in Glasgow on Thursday 31st October and Friday 1st November at the Hilton Doubletree hotel in Cambridge Street. The full conference programme will be announced shortly.

Read more

What we do - Making it crystal clear

Putting things in Perspective

The day to day work carried out by the members of the APA has a direct effect on our daily lives — from the food that we eat to the air we breathe, from checking the safety of children's toys to providing a frontline emergency response unit for chemical leaks.

Find out more

Case studies

View some recent case studies of work carried out by members of the APA showcasing the diverse range of instances where our members get involved.

Find out more

Job Vacancies

Tender Opportunities

Latest News

- Modern Apprentice from Glasgow Scientific Services scoops top award — 11 June 2019
- Fake Cheese? — 03 June 2019
- Toxic Chemicals in Everyday Life — 09 May 2019
- Visit by "Food Unwrapped" to Kent PA Laboratory — 05 May 2019
- Food: Truth or Scare — 22 February 2019

Laboratories

Find a lab

- Region - ▼ Search

...nalyst

The Journal of the Association of Public Analysts

Click to visit site

> 70 % of PA findings upheld

http://www.publicanalyst.com/

NML UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC

LGC

Answers

1. Inadequate planning for sampling
2. Incorrect sampling
3. Loss of chain of custody of sample
4. Inadequate method of analysis
5. Inadequate application of a method of analysis
6. Inadequate interpretation
7. Nature springs a nasty surprise
8. Poor reporting practice (allergens…)
9. Dated instrumentation
10. Inadequate bioinformatics

Inadequate planning for sampling

e.g. Planned sampling for food hypersensitivity …

Is the survey aimed to assess

(a) a gluten free meal for a person with coeliac condition? or

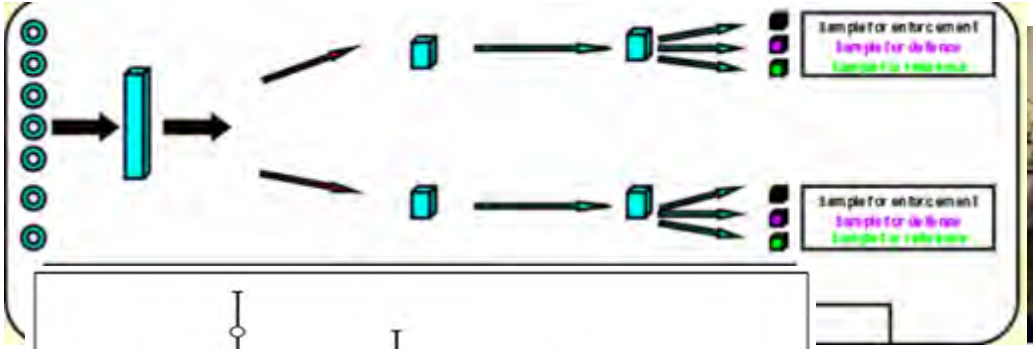
(b) a wheat-free meal for a person with wheat allergy? or

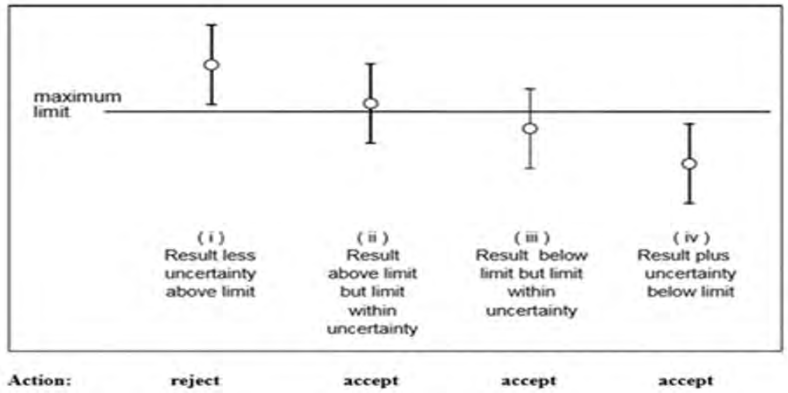
(c) both?

Result …'gluten ... 5 mg kg^{-1}' satisfactory if only (a) was the objective, but not if (b) was the objective, and (c) both - an opinion is required such as 'satisfactory with regard to the requirements for a food labelled as 'gluten-free' but may pose a risk to a person with wheat allergy'. This latter might be the trigger for a more in-depth look at the ingredients of the meal and a prompt to advise the business on the nuances of coeliac v's wheat allergy.

1. Leitch, I, Walker, M J, & Davey, R, 2005, Food Allergy: Gambling your life on a take-away meal, *Int. J. Environ. Health Res.* 2005, 15(2), 79 –87 ***2.*** McIntosh, J., Flanagan, Madden, Mulcahy, Dargan, Walker & Burns, 2011, Awareness of coeliac disease and the gluten status of 'gluten-free' food…in Ireland

Sampling / method / interpretation, e.g. Mycotoxins



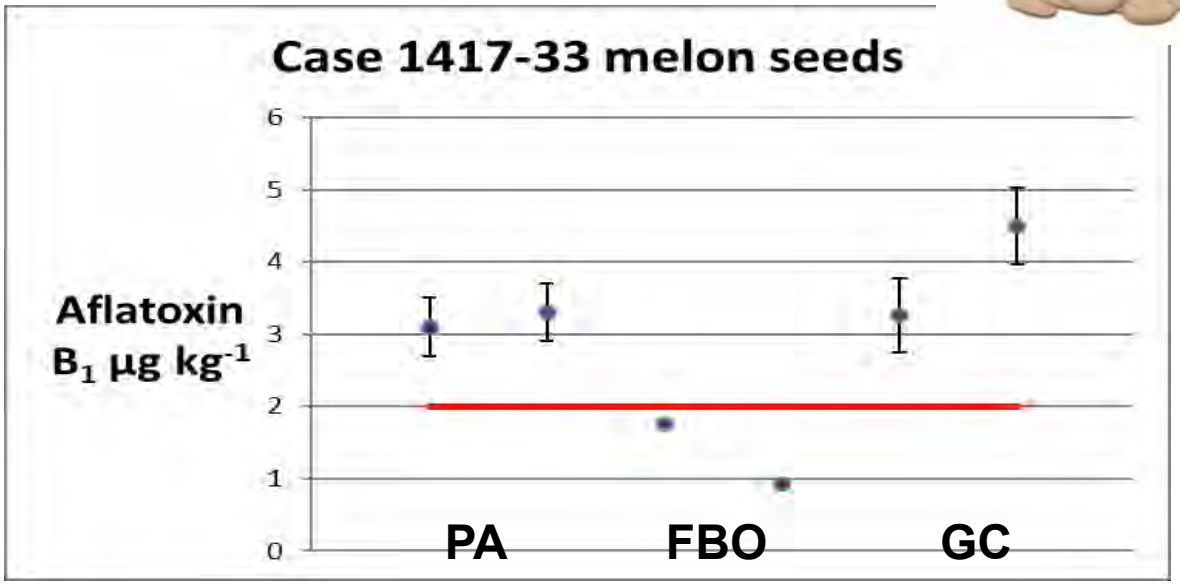


Trader's sample taken in 3rd country, Or … (UK) Lab forgets about nut to shell ratio, slurry ratio, recovery correction or measurement uncertainty

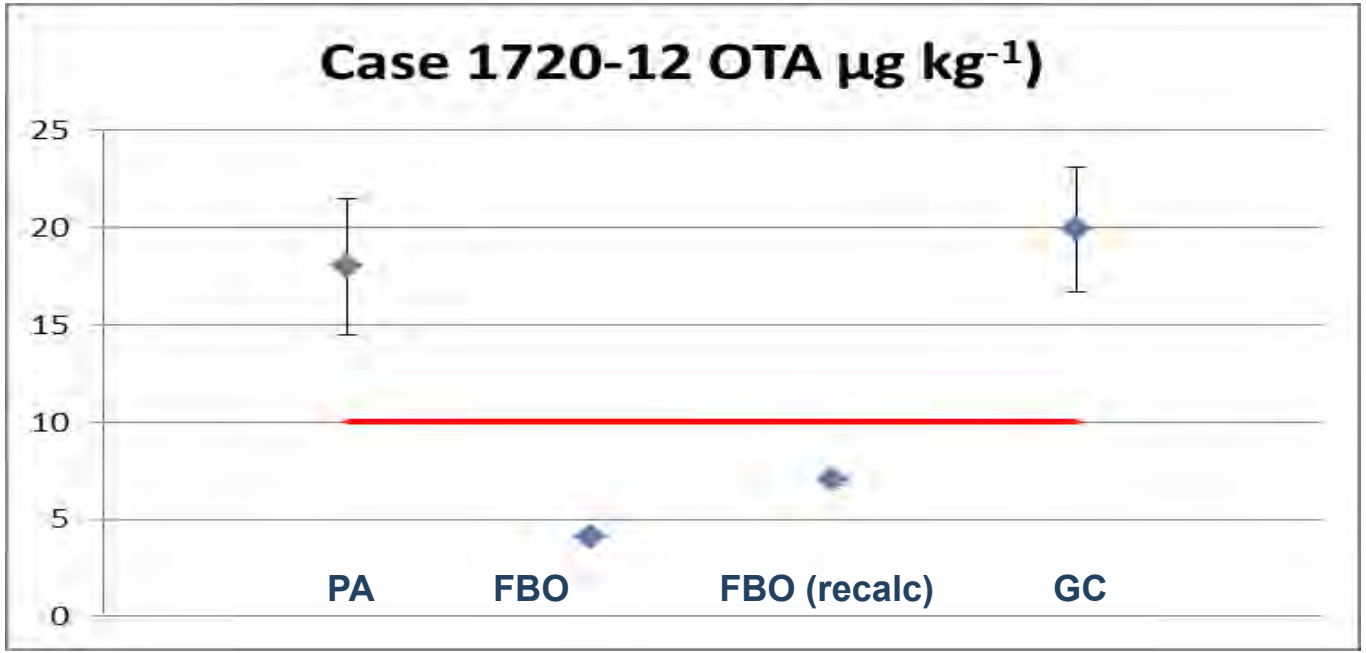
3. Walker, et al., 2017, Aflatoxins in Groundnuts – Assessment of the Effectiveness of EU Sampling and UK Enforcement Sample Preparation Procedures, *J Assoc Public Analysts*, 45, 1 – 22

Melon seeds – “Agushi”

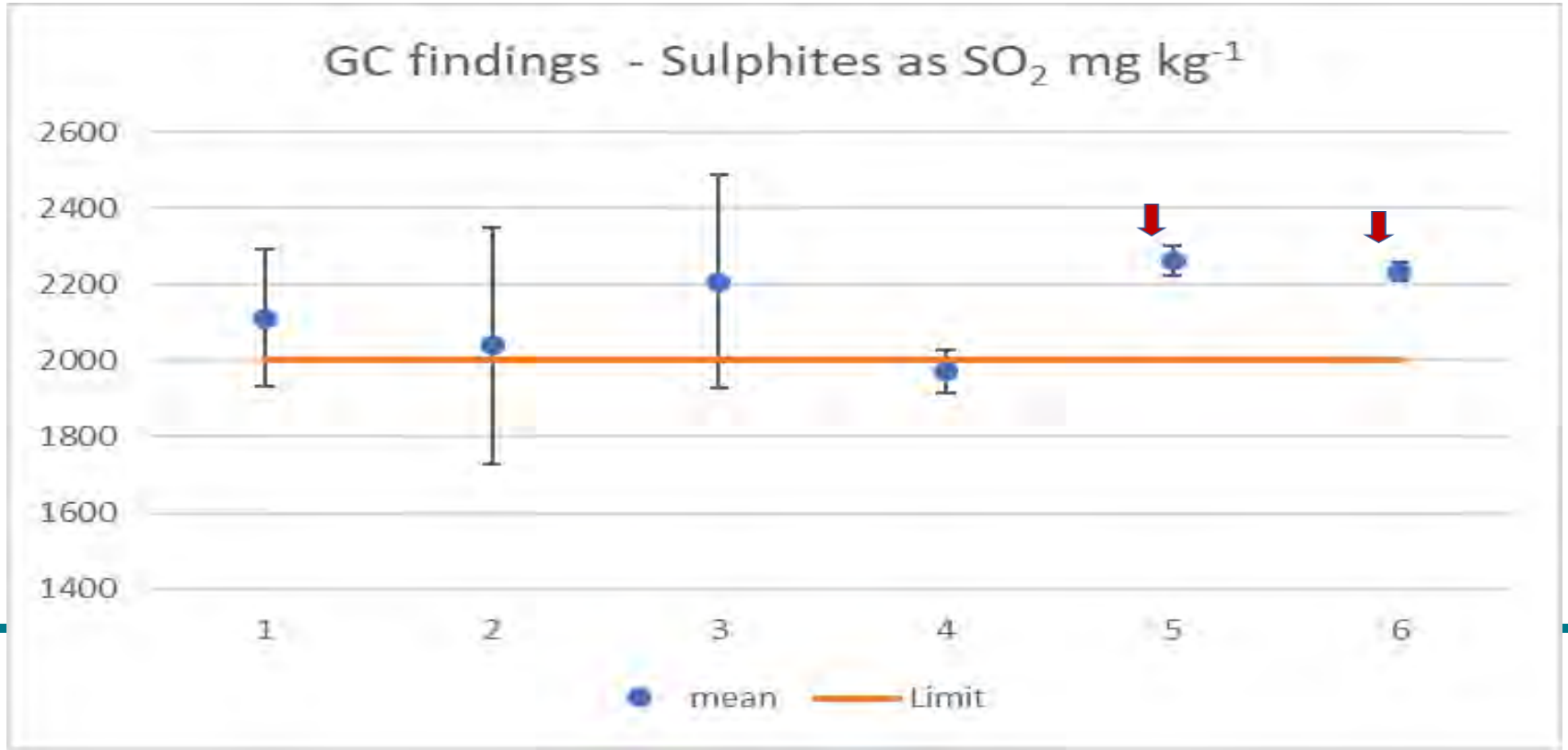
One case – 2 samples



Results for Case 1720-12 OTA

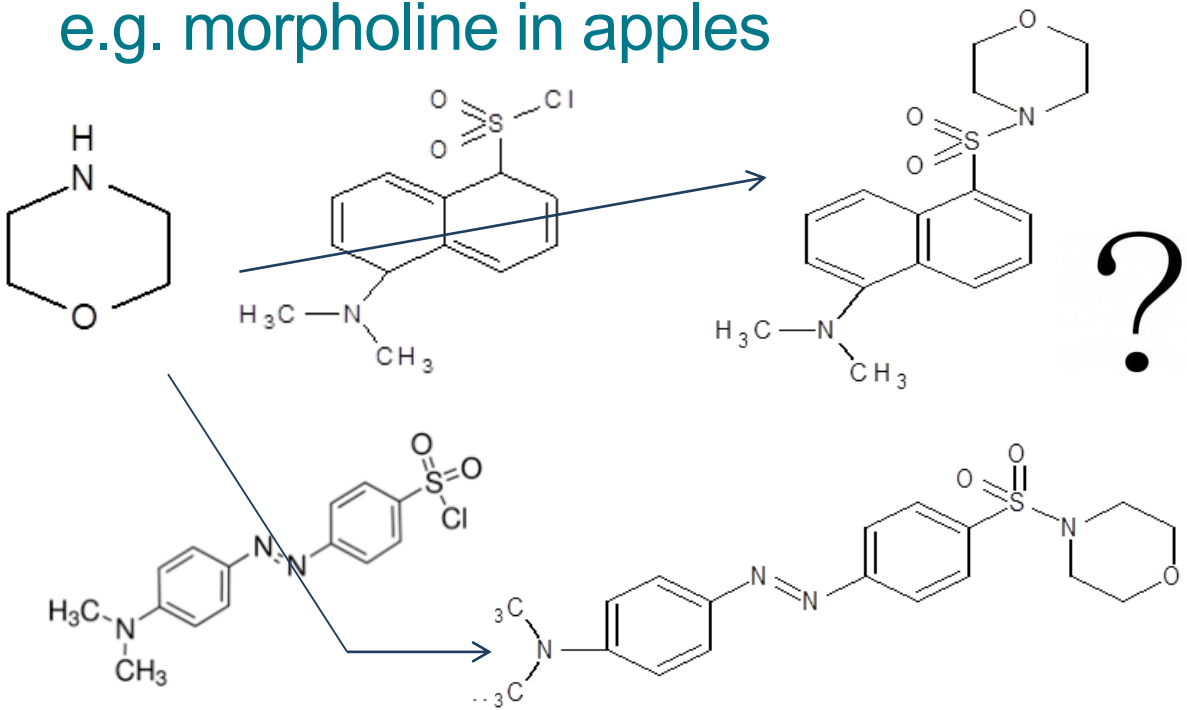


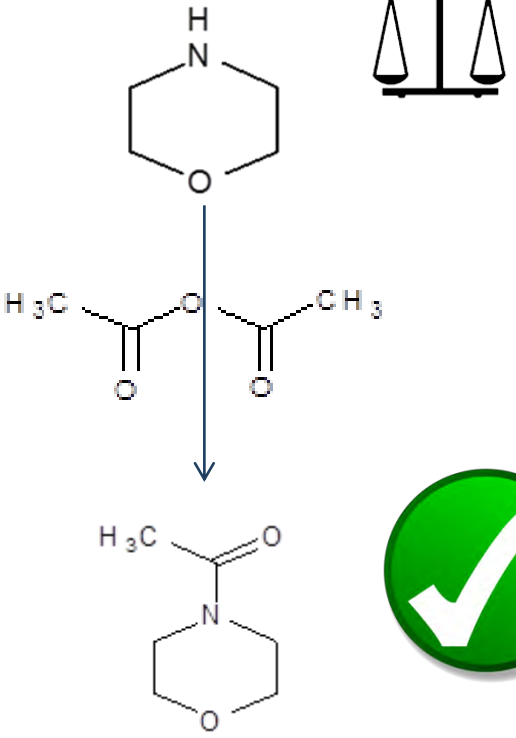
Sulphur dioxide in apricots



Inadequate method of analysis

e.g. morpholine in apples





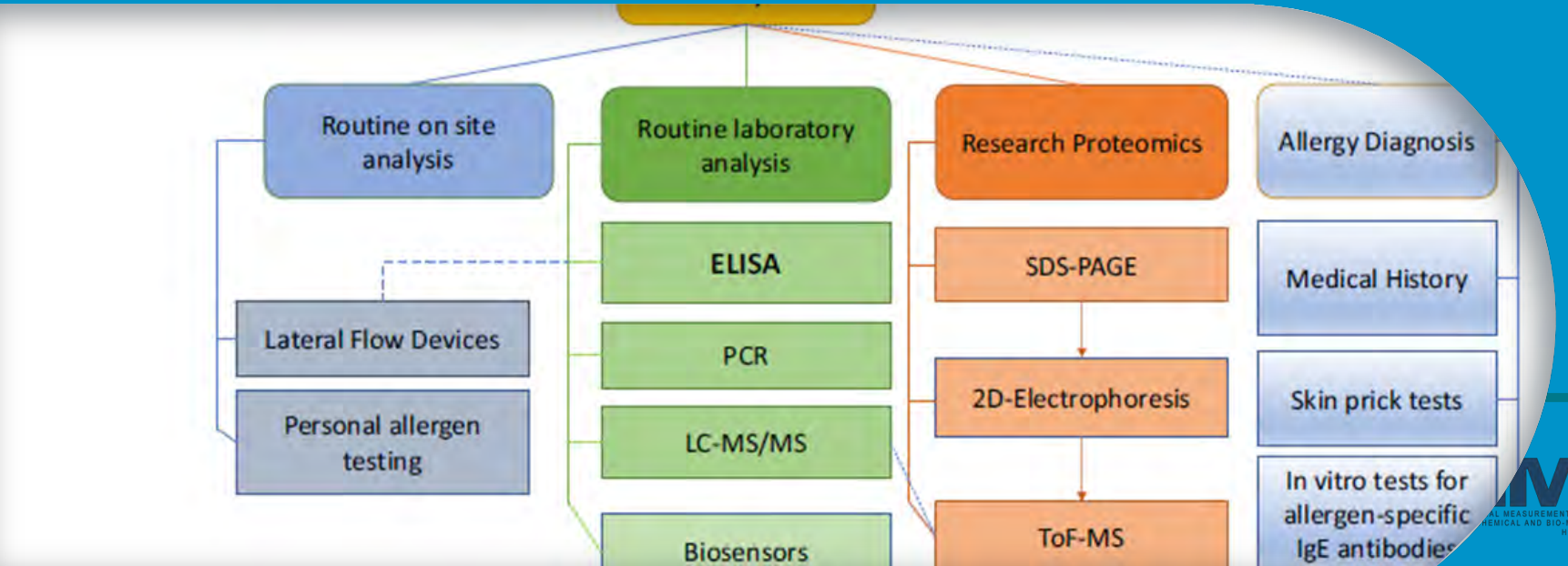
4. Michael J. Walker, Kirstin Gray, Christopher Hopley, David Bell, Peter Colwell, Peter Maynard and Duncan Thorburn Burns, 2011, Forensically Robust Detection of the Presence of Morpholine in Apples—Proof of Principle

Nature springs a nasty surprise

Nitrofurans

Almond Mahaleb

Mānuka honey



Nitrofurans - SEM

Parent drug	Marker metabolite	Abbreviation
Furazolidone	3-amino-oxazolidinone	AOZ
Furaltadone	3-amino-5-morpholinomethyl-1,3-oxazolidinone	AMOZ
Nitrofurantoine	1-aminohydantoin	AHD
Nitrofurazone	Semicarbazide	SEM

5. John Points, D. Thorburn Burns, Michael J. Walker, 2014, Forensic issues in the analysis of trace nitrofuran veterinary residues in food of animal origin, Food Control, 50, 92-103

Almond or mahaleb – cumin & paprika recalls

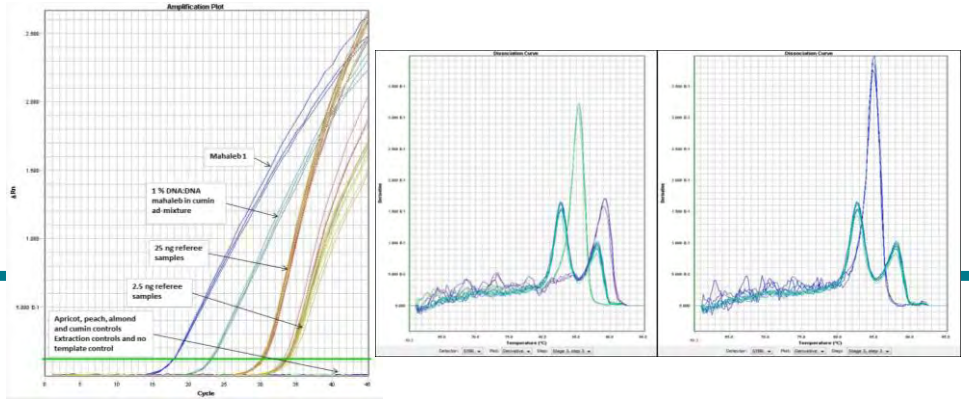
ELISA

PCR

LC-MS/MS for Prunus Species-specific peptides

qPCR assay for Mahaleb

PCR screening assay



Peptide	Precursor Ion /m/z		Almond	Mahaleb
FVSSMLR	2+	420.2258		
SGGQILPIR	2+	470.7824		
DFVSSPFR	2+	477.7376		
DRLVASVDLPLLR	3+	489.6278		
VPTPVPPRVSSPR	2+	694.9041		
ALPDEVLANAYQISREQAR	4+	536.7828		
ALPDEVLANAYQISR	2+	830.4387		
VQGQLDFVSPFRS	2+	740.3832		
TEENAFINTLAGR	2+	718.3624		
ISTLNSHNLPILR	3+	493.2877		
GNLDFVQPPR	2+	571.8013		
GVLGAVFSGCPETFEESQQSSQQGR	3+	895.7452		

NIVIL UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC

LGC

Papers

5. Burns, M., Walker, M., Wilkes, T., Hall, L., Gray, K. and Nixon, G. (2016) Development of a Real-Time PCR Approach for the Specific Detection of *Prunus mahaleb*. *Food and Nutrition Sciences*, 7, 703-710.

6. Nixon, G., Hall, L., Wilkes, T., Walker, M. and Burns, M. (2016) Novel Approach to the Rapid Differentiation of Common *Prunus* Allergen Species by PCR Product Melt Analysis. *Food and Nutrition Sciences*, 7, 920-926.

7. Walker, M.J., Burns, D.T., Elliott, C.T., Gowland, M.H. and Mills, E.C., (2016), Is food allergen analysis flawed? Health and supply chain risks and a proposed framework to address urgent analytical needs. *Analyst*, 141(1), pp.24-35

8. Inman, S.E., Groves, K., McCullough, B., Quaglia, M. and Hopley, C., 2018. Development of a LC-MS method for the discrimination between trace level Prunus contaminants of spices. *Food chemistry*, 245, pp.289-296.

9. Walker, M.J., Burns, M., Quaglia, M., Nixon, G., Hopley, C.J., Gray, K.M., Moore, V., Singh, M. and Cowen, S., (2017), Almond or Mahaleb? Orthogonal Allergen Analysis During a Live Incident Investigation by ELISA, Molecular Biology, and Protein Mass Spectrometry. *Journal of AOAC International* ,101, 162 - 169

Authenticity of Mānuka honey
- determination of exogenous sugars

$\delta^{13}C$ ‰

$\delta 13C_{CHO} \sim \delta 13C_{protein}$

C_3 e.g. Honey
-33 ……-22

-28 .. -23

C_4 e.g. sugar cane, corn syrup
-16 ……. -8

-15 …-9

CAM*
e.g. agave
-20 ……-10

Carter, J.F. and Chesson, L.A. eds., 2017. Food Forensics: Stable Isotopes as a Guide to Authenticity and Origin. CRC Press.

*crassulacean acid metabolism

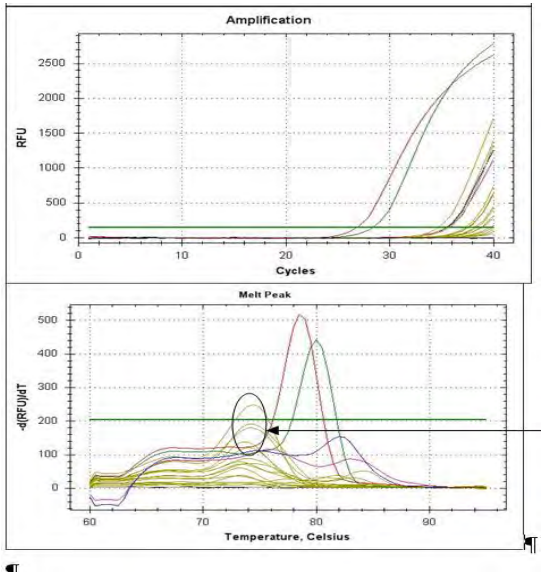
NML UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC

LGC

Reporting the results of allergen analysis

- **Method of analysis – ELISA, PCR or LC-MS/MS**
- ***[X] mg/kg as Y*,**
  - where [X] is the best estimate of the concentration of allergen found by analysis of the sample received after in-laboratory homogenisation, extraction and analysis by a validated method, and
  - Y is EITHER the allergen protein OR the name of the food.
- **But if the whole food is the reporting basis the conversion factor from allergen protein to whole food must be given.**
- **Conversion factors should be agreed with literature references to the typical protein contents of (at least) Annex II allergens. Adding the N to protein factor would be useful.**
- ***As a matter of routine the basis of data as allergen or (preferably) allergen protein should be specified every time a datum is given in a method or report.***

Instrumentation – GMO detection - rice



DNA sequences -

1. 35S promoter from Cauliflower Mosaic Virus (P35S)
2. Nopaline synthase terminator (TNOS) derived from *Agrobacterium tumefaciens*
3. Genetically engineered CryIAb/CryIAc

1. BIO-RAD CFXTM Real-Time PCR System
2. Applied Biosystems™ 7900HT Fast Real-Time PCR System
3. Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System

Inadequate bioinformatics

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]”

Squid - dispute

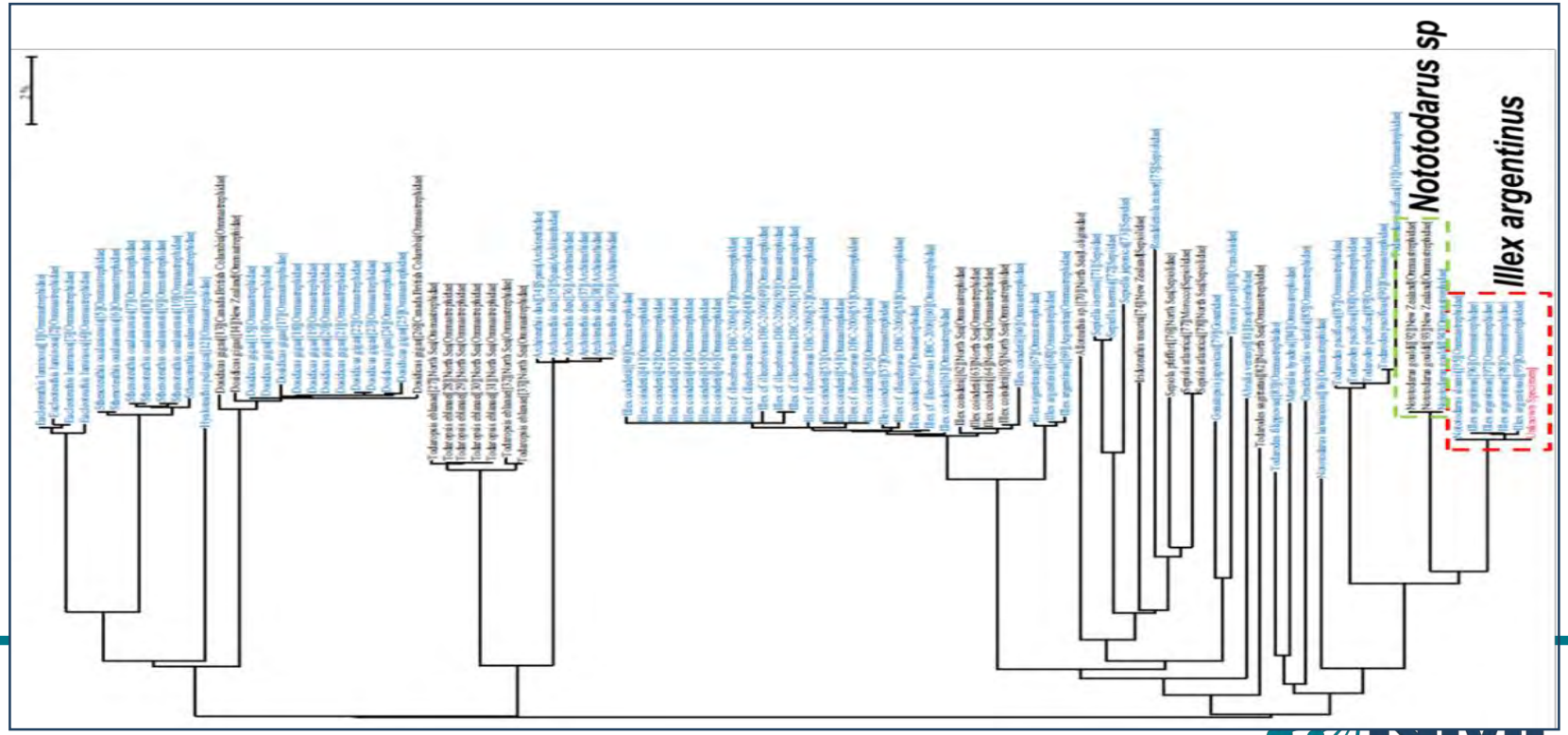
Arrow squid is the commercial designation for squid of the species Nototodarus gouldi and Nototodarus sloani

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

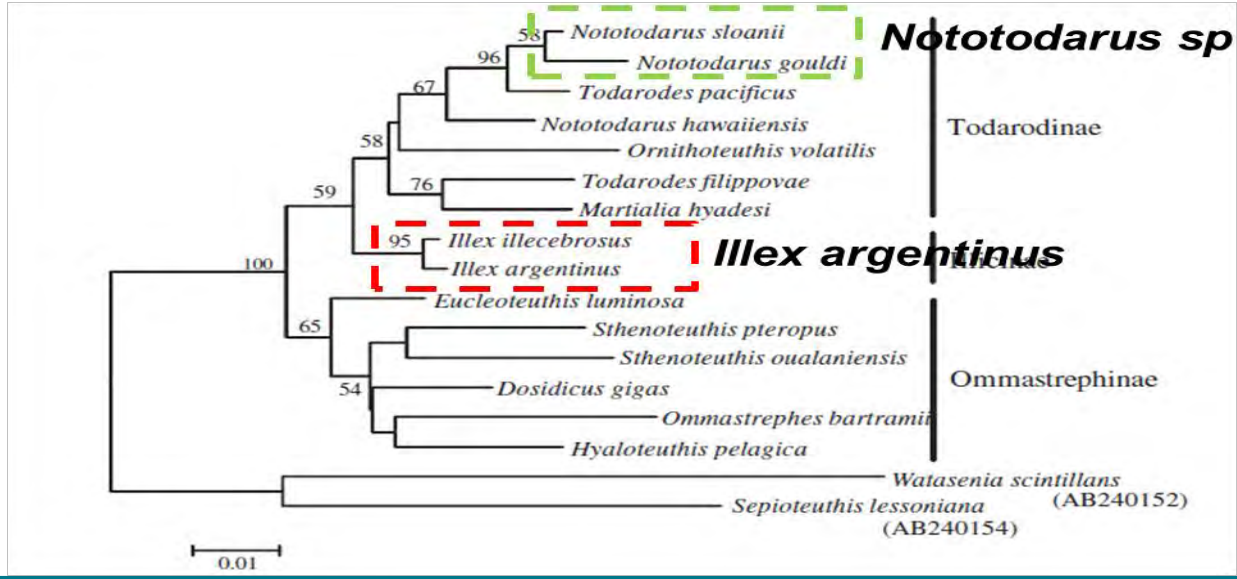
Phylogenetic tree Ommastrephidae

differentiation by COI gene data available in '*BOLD*'



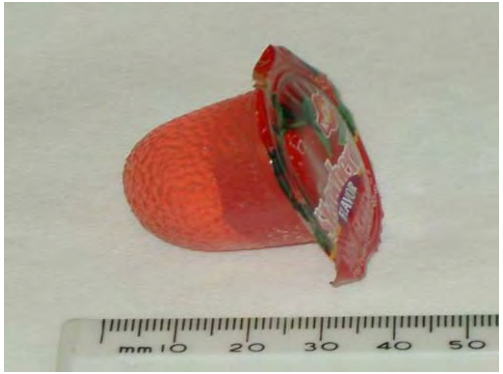
Phylogenetic tree Cephalopoda

differentiation by 16s rRNA sequence in GenBank



Conclusions – squid case

- 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.
- Public Analyst and FBO labs 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

Jelly confectionery: a choking hazard?

Evaluation and assessment of jelly mini-cups – workshop

Wednesday 13 March 2019

LGC, Queens Road, Teddington
Middlesex, TW11 0LY

Building on previous joint Defra, FSA, FSS and Government Chemist Knowledge Transfer (KT) events delivered by LGC, this workshop is one in a new series of government-funded events. It will focus on providing advice, guidance and practical knowledge on assessing the conformity of confectionery items to the Regulation definition of jelly mini-cups.

Background

There have been several instances worldwide of children and elderly people choking on soft slippery dome-shaped jellies that are designed to be consumed in one bite. Food additive law, Regulation (EC) No 1333/2008, provides a definition of jelly mini-cups and contains provisions to address choking risks posed by such items. Although the definition seems straightforward, it poses several difficulties, for example what does 'firm consistence' mean and how should 'intended to be ingested in a single bite ...' be interpreted? Disputes and requests for advice in this area continue to be a feature of the Government Chemist's work.

NML UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC

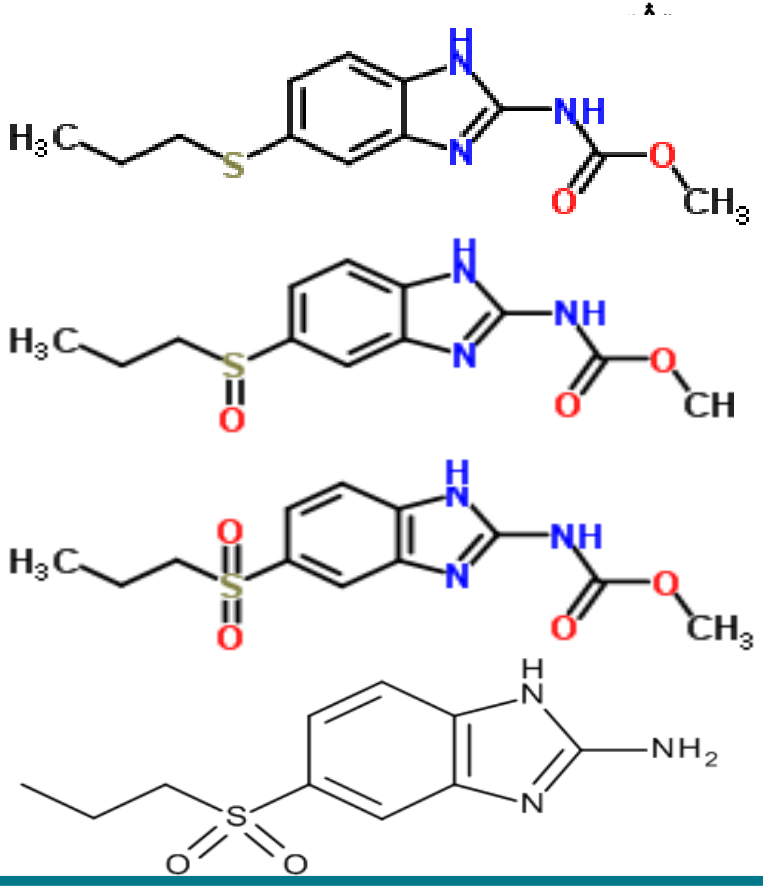
LGC

Veterinary Residues

Albendazole in consignment of corned beef at UK Port from Brazil

Albendazole

- Benzimidazole anthelmintic used in ruminants, rapidly metabolised
- MRL in muscle, fat 100 µg kg^{-1} as the sum of albendazole sulphoxide, albendazole sulphone, and albendazole 2- amino sulphone, expressed as albendazole
- RASFFs



Initial contact ...

- PHA – consignment failed for albendazole, owner's portion analysed satisfactory,
- " ... no retained portion of the formal sample..."
- PA found 245 ± 65 µg kg^{-1} albendazole as the MRL definition
- FBO lab reported '< MRL' ..... Further enquiry ...
- Albendazole 80 µg kg^{-1} , albendazole sulfoxide 82 µg kg^{-1}
- But "… no retained unopened cans...."
- We agreed to re-analyse the previously analysed homogenates from each lab but also requested a new sampling exercise .....
- Consignment 54,000 cans (340 g), 8.36 tonnes, two production dates
- $3\sqrt{}$ [54,000] ~ 38, hence requested 20 cans randomly from each production date
- But when labs forwarded their samples turned out there were unopened cans ...

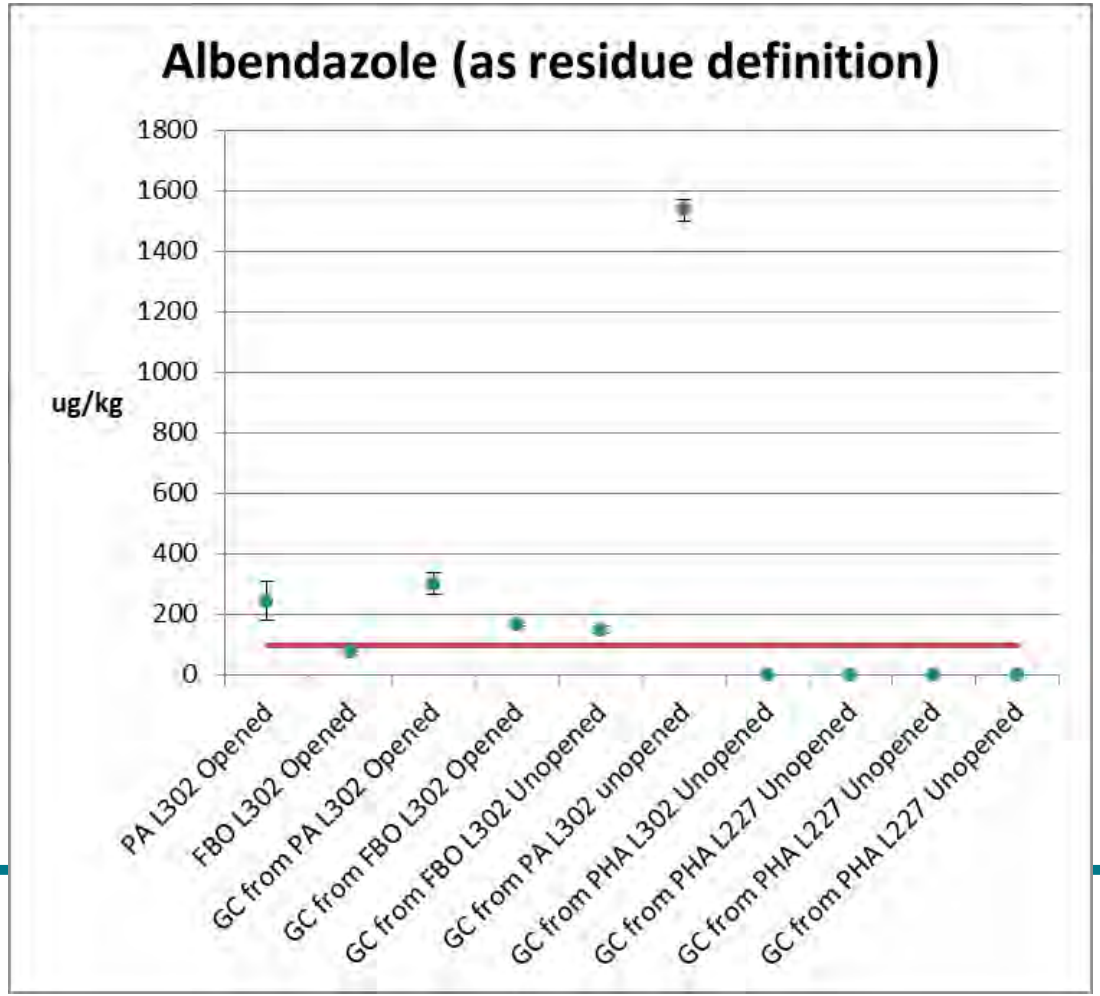
What was analysed

LGC sample reference	Received from	Lot number	Opened / unopened	PA µg kg^{-1}	FBO µg kg^{-1}
826389	FBO	150302	Unopened		
826390	FBO	150302	Opened		77 ± ?
826391	PA	150302	Unopened		
826392	PA	150302	Opened	245 ± 65	
826393	PHA	150302	Unopened		
826394	PHA	150227	Unopened		
826395	PHA	150227	Unopened		
826396	PHA	150227	Unopened		

Analysis

- Acetonitrile extraction, liquid / liquid partitioning, SPE clean-up
- LC-MS/MS
- Isotopically labelled albendazole D3 and albendazole sulphoxide D3 were used as internal standards.
- Two precursor ion to product ion transitions each analyte
- Quantification against calibration curves established by a series of pre-extraction matrix standards

- Only the sulphoxide was found

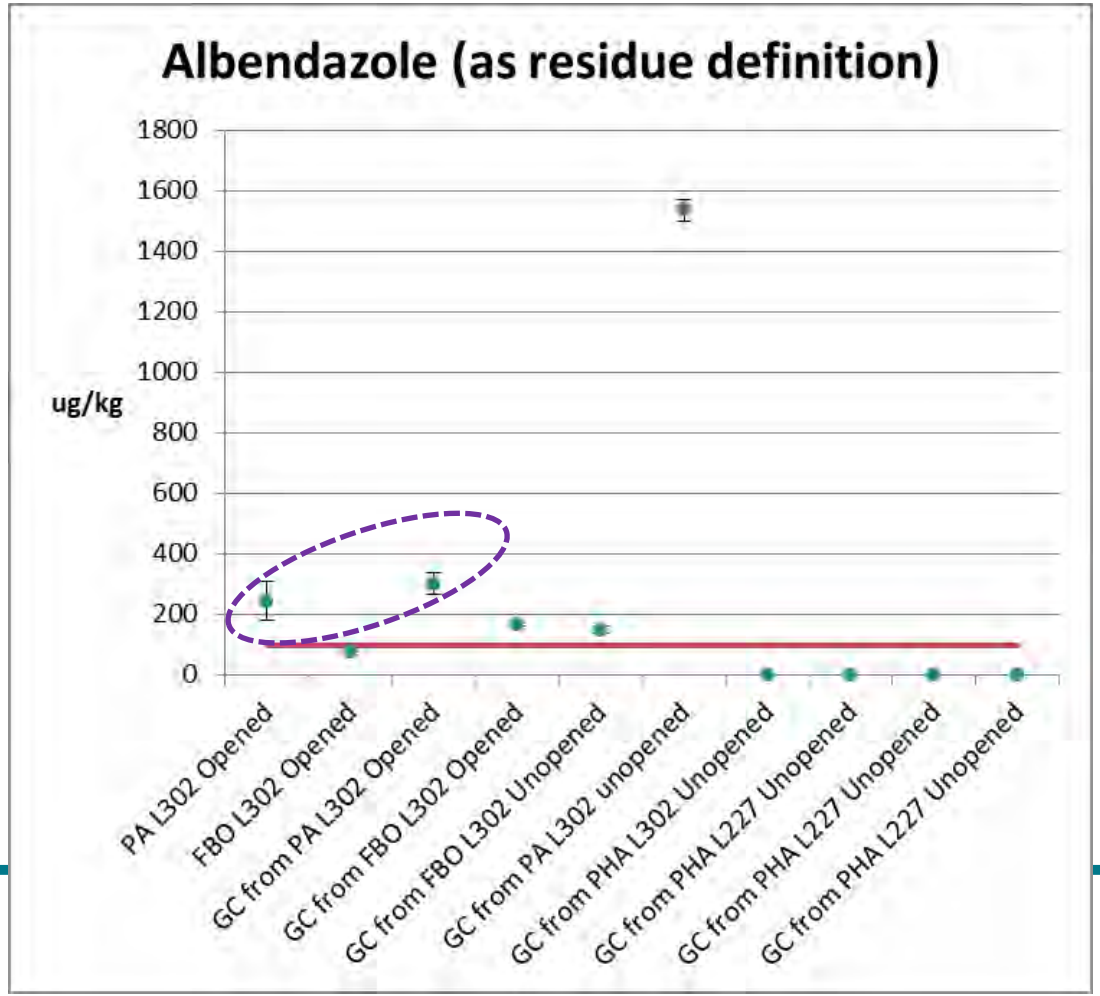


Residue definition

MRL in muscle / fat:-
100 µg kg-1 as the sum of albendazole sulphoxide, the sulphone, and the 2-amino sulphone,

expressed as albendazole

NML
UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC
LGC

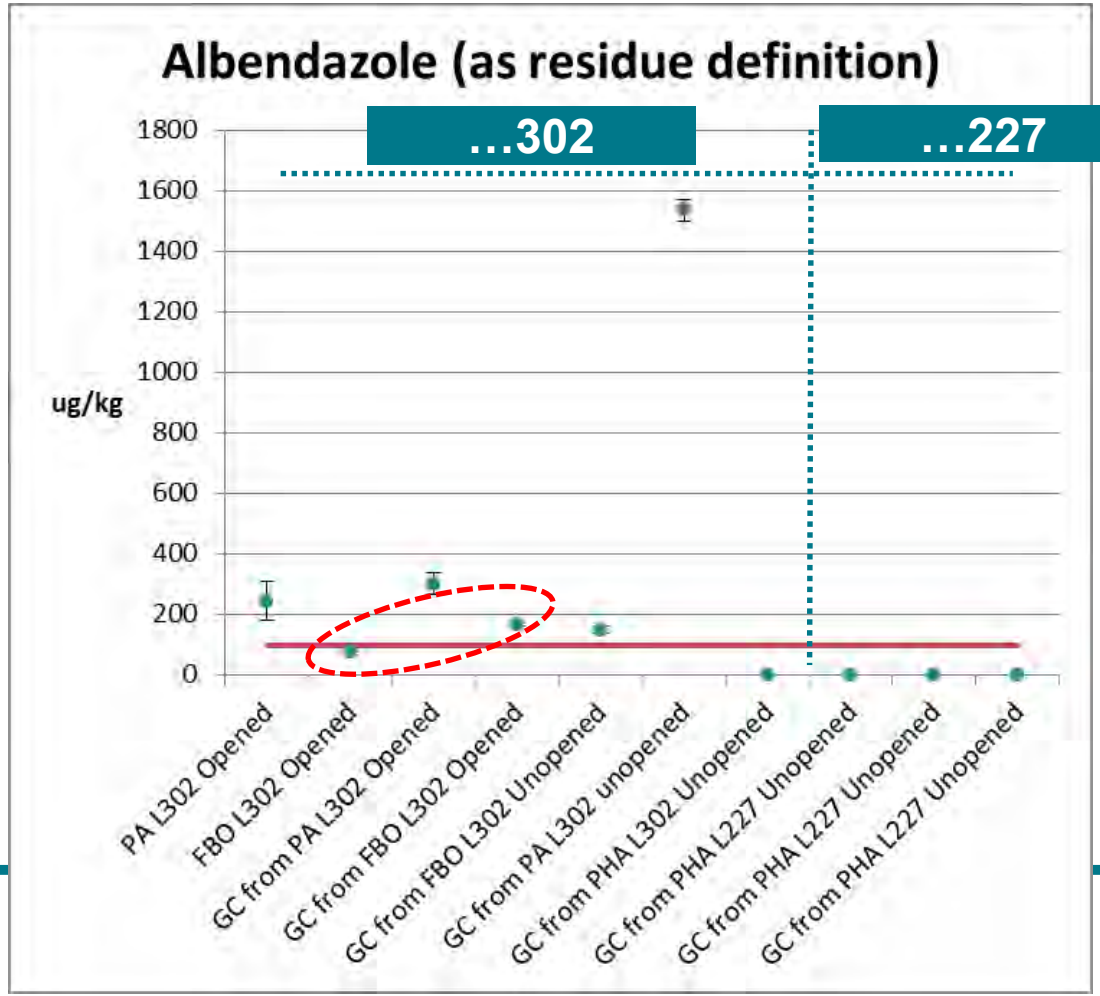


Residue definition

MRL in muscle / fat:-
100 µg kg-1 as the sum of albendazole sulphoxide, the sulphone, and the 2-amino sulphone,

expressed as albendazole

NML
UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC
LGC



← Denotes different date of production

Residue definition

MRL in muscle / fat:-
100 µg kg-1 as the sum of albendazole sulphoxide, the sulphone, and the 2-amino sulphone,

expressed as albendazole

Interpretation (as of 2016)

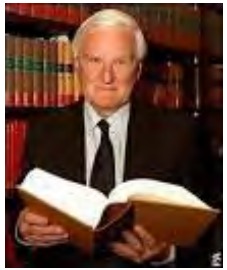
- Reg 37/2010 allows albendazole in ruminants but limits the residues to 100 µg kg^{-1}
- Art. 23 of Reg 470/2009 - if >MRL … non-complaint with Community legislation
- Art.14 (6) of Reg 178/2002 ... where any food which is unsafe is part of a batch, lot or consignment of food of the same class or description, it shall be presumed that all the food in that batch, lot or consignment is also unsafe, unless following a detailed assessment there is no evidence that the rest of the batch, lot or consignment is unsafe

Two production dates …

- Art.14 (7) of Reg (EC) No 178/2002 … food that complies with specific Community provisions … shall be deemed to be safe.

- Hence ..... albendazole >MRL beyond reasonable doubt, does not comply .. is unsafe and the consignment cannot be placed on the market ..... *BUT* …

- JR of previous OVS decision ….



FOOD ADDITIVES & CONTAMINANTS: PART A, 2016
http://dx.doi.org/10.1080/19440049.2016.1243807

Taylor & Francis
Taylor & Francis Group

Resolution of a disputed albendazole result in the UK Official Control System – time for more guidance?

Michael Walker[a], Kirstin Gray[a], Christopher Hopley[b], Christopher Mussell[b], Louise Clifford[c], Jayanie Meinerikandathevan[c], Leonardo Firpo[c], Joanna Topping[c] and Daniel Santacruz[d]

[a]Government Chemist Programme, LGC, Teddington, UK; [b]Science & Innovation Division, Teddington, UK; [c]Laboratory and Managed Services, LGC, Teddington, UK; [d]London Port Health Authority, Stanford-Le-Hope, Essex, UK

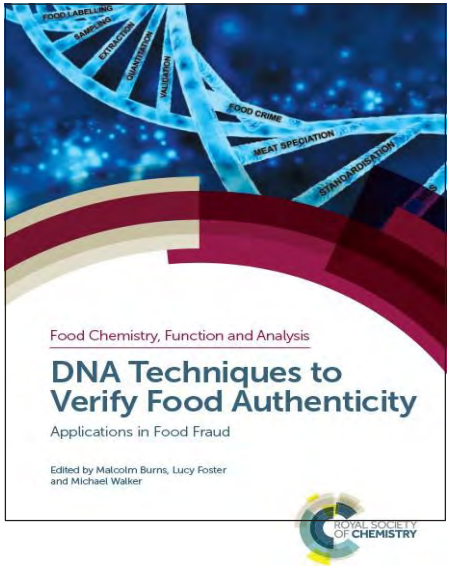
ABSTRACT

Albendazole, one of the benzimidazole anthelmintics, is used in ruminants and has maximum residue limits in muscle, fat and other tissue owing to reported teratogenicity. Albendazole is extensively metabolised in domestic animals and humans with rapid conversion to a sulphoxide and subsequently sulphone and amino sulphone metabolites. Sulphoxide metabolites are responsible for the systemic biological activity of benzimidazole drugs. Herein we report a case of disputed results for albendazole in a consignment sampled at import in which the Official Analyst certified against the consignment for excess albendazole. A laboratory acting for the importer reported data below the MRL, including a finding of the parent drug which is not included in the residue definition. The Government Chemist has a statutory duty as a route of technical appeal in the UK Official Food Control system and the case was referred for referee analysis. We report our findings based on a LC-MS/MS method, which confirmed the official findings, did not reveal the presence of the parent drug but identified hot spots of albendazole marker residues in the consignment. We discuss the need for recommendations on official sampling at import and interpretation of results.

ARTICLE HISTORY
Received 24 August 2016
Accepted 28 September 2016

KEYWORDS
Albendazole; technical appeal; sampling; law; corned beef

1. Inadequate planning for sampling - allergens
2. Incorrect sampling - mycotoxins
3. Loss of chain of custody of sample
4. Inadequate method of analysis – morpholine
5. Inadequate application of a method of analysis
6. Inadequate interpretation - mycotoxins
7. Nature springs a surprise – SEM, mahaleb, … manuka honey SCIRMS
8. Poor reporting practice (allergens…)
9. Dated instrumentation – trace stochastic GMO
10. Inadequate bioinformatics – squid (but also plant allergens …)



DNA Techniques to Verify Food Authenticity

Applications in Food Fraud

Malcolm Burns *LGC Limited, UK*
Lucy Foster *DEFRA, UK*
Michael Walker *Michael Walker Consulting Ltd, UK*

Hardback | 320 | 9781788011785 | £149.00 | $205.00 | 26/09/2019

Published October 2019

https://doi.org/10.1039/9781788016025

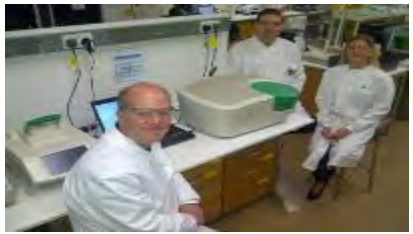
NML UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC
LGC










NML
UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC

LGC

Acknowledgements

Gill Holcombe
Kirstin Gray
Malcolm Burn
Malvinder Singh
Magdalena Mazur
Andrew Campbell
Malcolm Burns,
Gavin Nixon & team

Steve Ellison
Simon Cowen
Phillip Wilson
Milena Quaglia
Kate Groves
Bryan McCullough
Sophie Inman
Luis Ruano Miguel

Department for Business, Energy & Industrial Strategy
FUNDED BY BEIS

Department for Environment Food & Rural Affairs

Food Standards Agency
food.gov.uk

Food Standards Scotland
For safe food and healthy eating

NML
UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIO-MEASUREMENT HOSTED AT LGC

LGC

Thank you for listening …

michael.walker@lgcgroup.com