Why do labs get it wrong?
Lessons from Referee Cases

Michael Walker

24 June 2020

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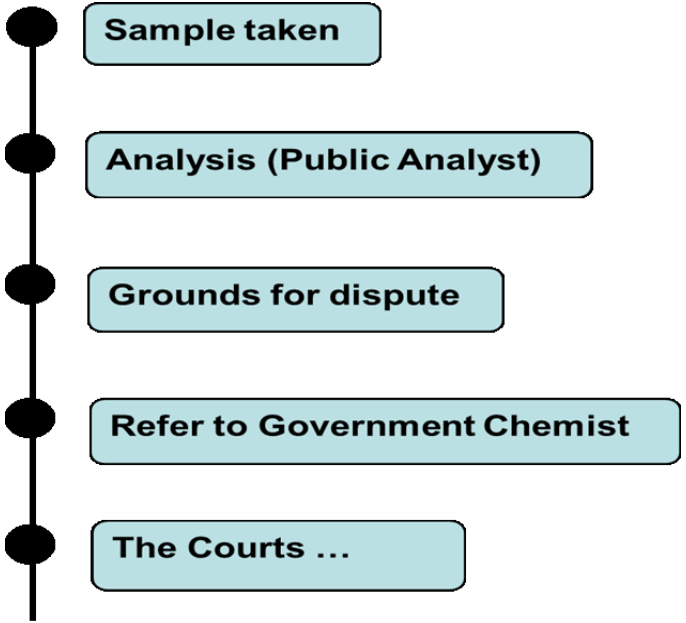
Government Chemist acts …



- 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

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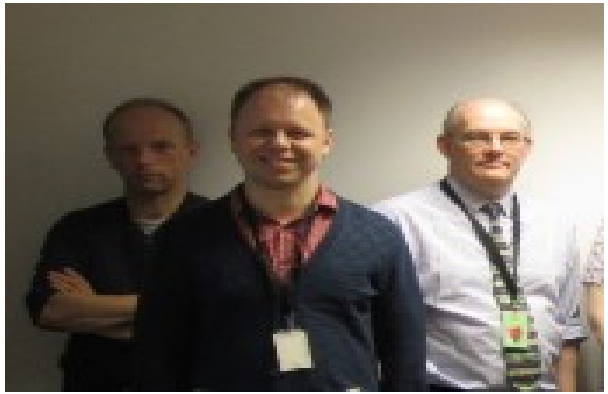
Typical steps in a referee case…

- **Sample receipt**
  1. Accept referral?
  2. Funding
  3. Schedule work
  4. Check legislation
  5. Identify method

Typical steps in a referee case…

- **Sample analysis**
  6. Investigate method
  7. Replicates 3 x 3
  8. CRMs, RMs spikes
  9. Witnessed
  10. Orthogonal confirmation if possible …
  11. Minuted meetings

Typical steps in a referee case…



- **Data analysis**
  12. Transcriptions checked
  13. Results reviewed
  14. New analytical runs if required
  15. Statisticians review dataset

Typical steps in a referee case…

- **Reporting**

15. Certificate drafted
16. Reviewed
17. Data independently checked
18. **Peer review**
19. Certificate issued to all parties

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

Why do laboratories get it wrong?

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 or reporting
7. Nature springs a surprise
8. Inadequate bioinformatics

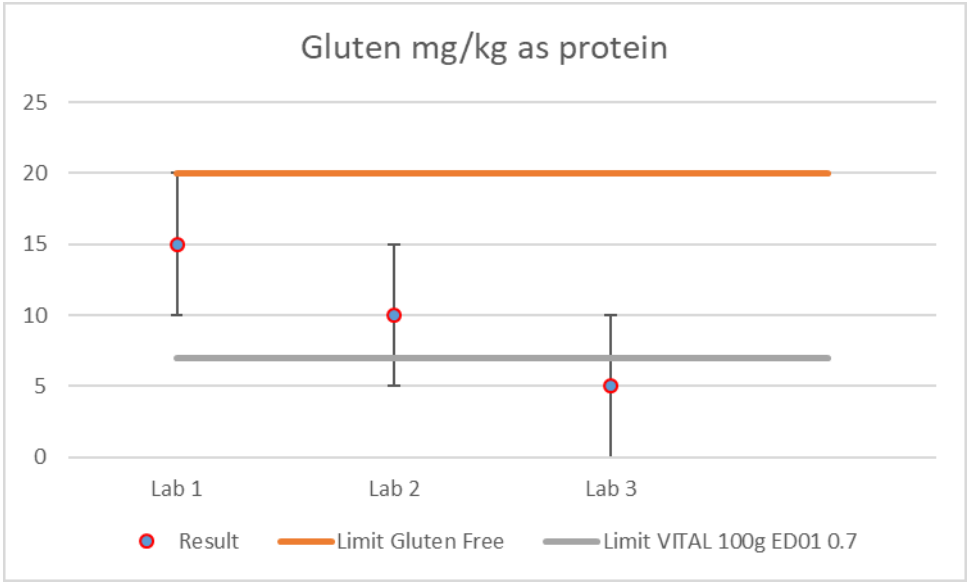
1. Inadequate planning for sampling - e.g. planned sampling for food hypersensitivity …

Is the survey aimed to assess

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

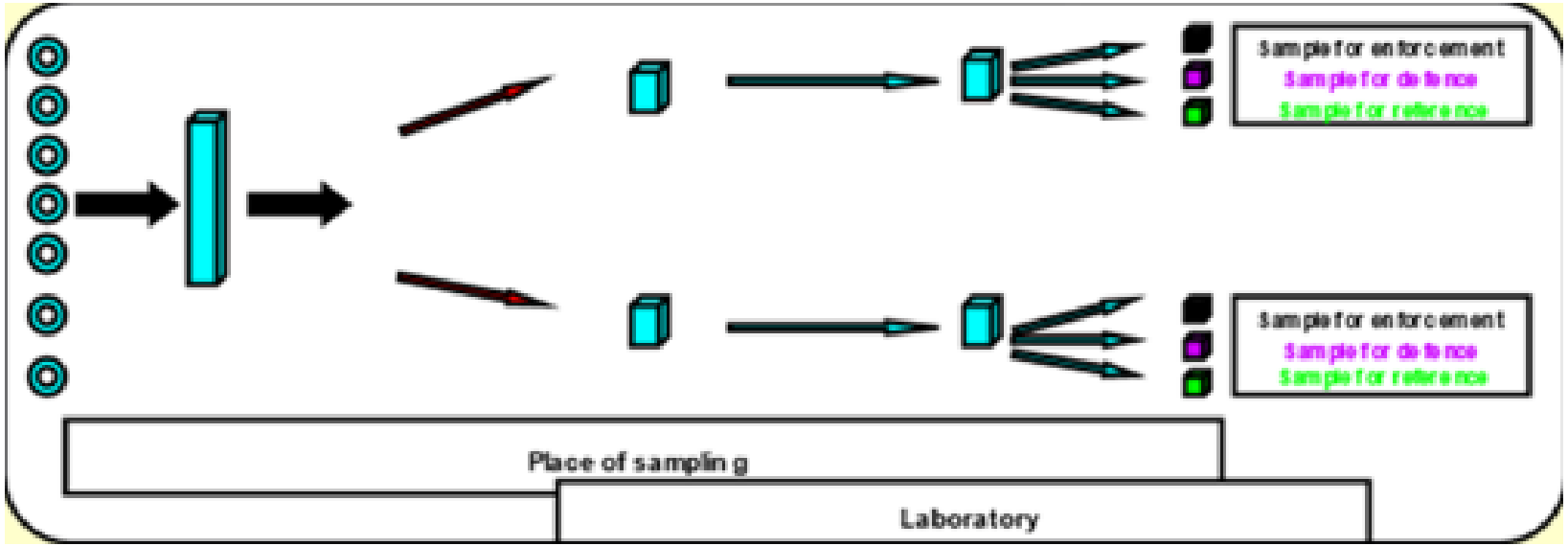
(b) a wheat-free meal for a person with wheat allergy? or

(c) both?



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 , *Int. J. Food Science & Technology*. 46, 1569–1574

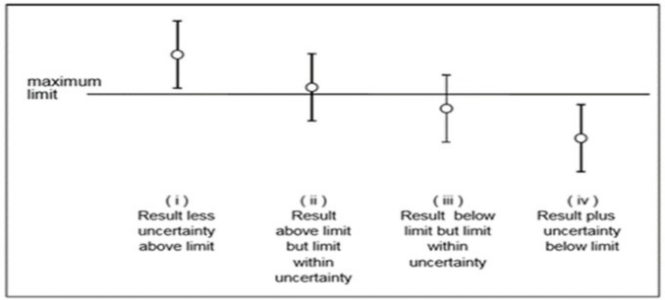
2. Sampling



3. Walker, Colwell, Cowen, Ellison, Gray, Elahi *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

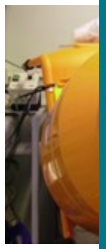
5. Inadequate application of a method interpretation, e.g. Mycotoxins

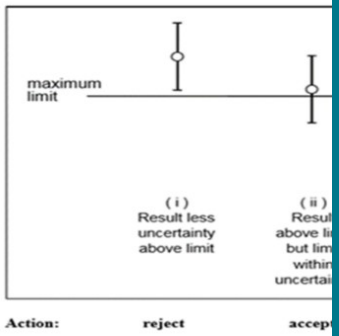
6. Inadequate



$$R = \left(m_{total} - m_{native}\right) / m_{spike}$$

3. Walker, Colwell, Cowen, Ellison, Gray, Elahi *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





Sample taken in country of export compliant
- Sampling?
- Mould proliferation in transit?

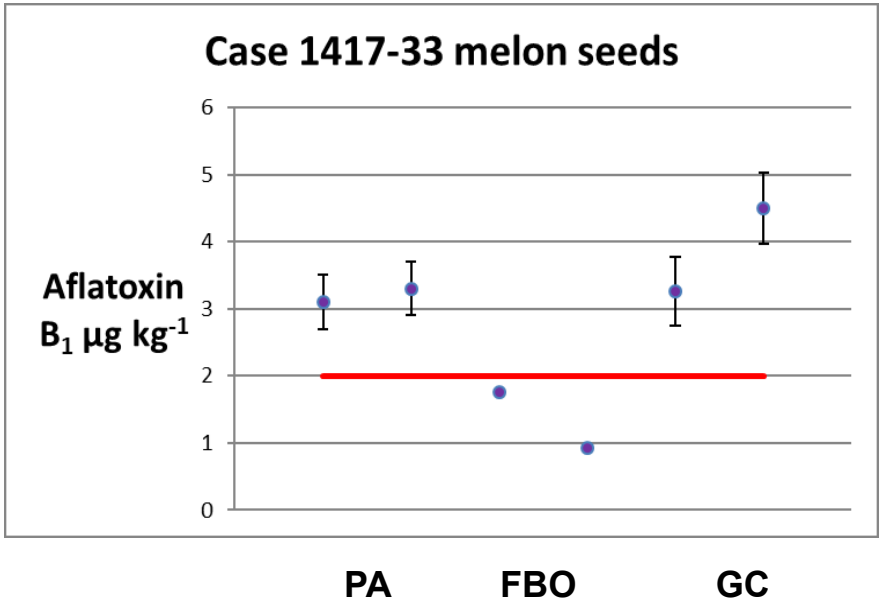
Or … (+ UK)

Lab forgets about
- Nut to shell ratio,
- Slurry ratio,
- Recovery correction,
- Measurement uncertainty … or
- All of the above?

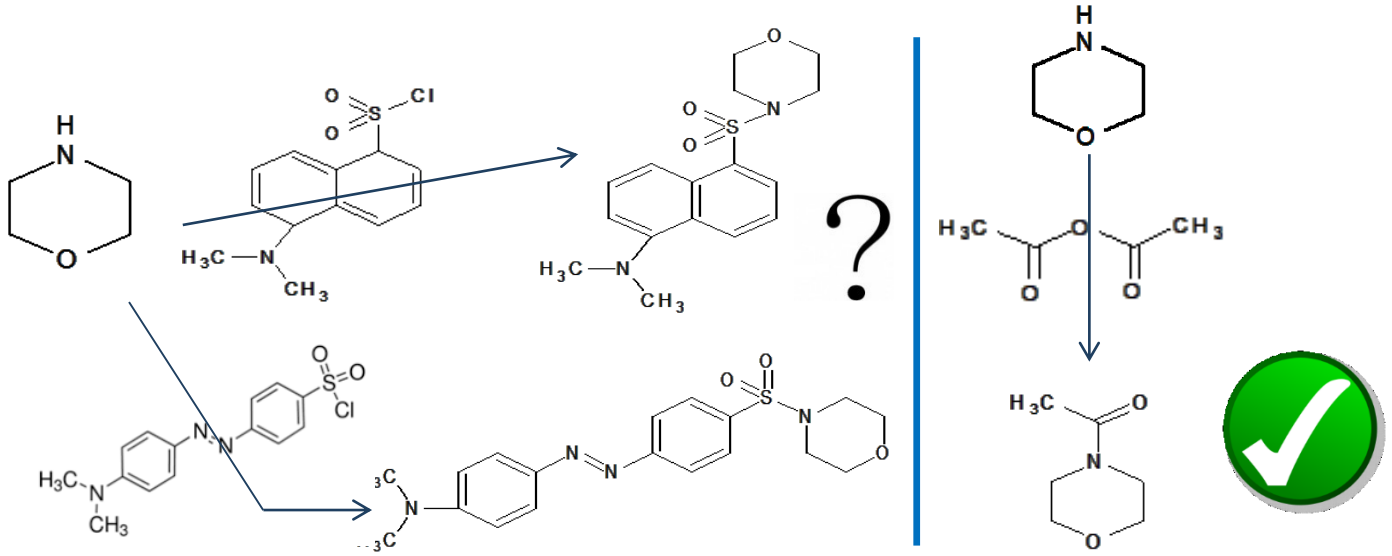
3. Walker, Colwell, Cowen, Ellison, Gray, Elahi *et al*., 2017, Aflatoxins in Groundnuts – Assessment of the Effectiveness of EU Sampling and UK Enforcement Sample Preparation Procedures, *J Assoc Public Analysts*, 45, 1 – 22

Melon seeds – “Agushi”

One case – 2 samples



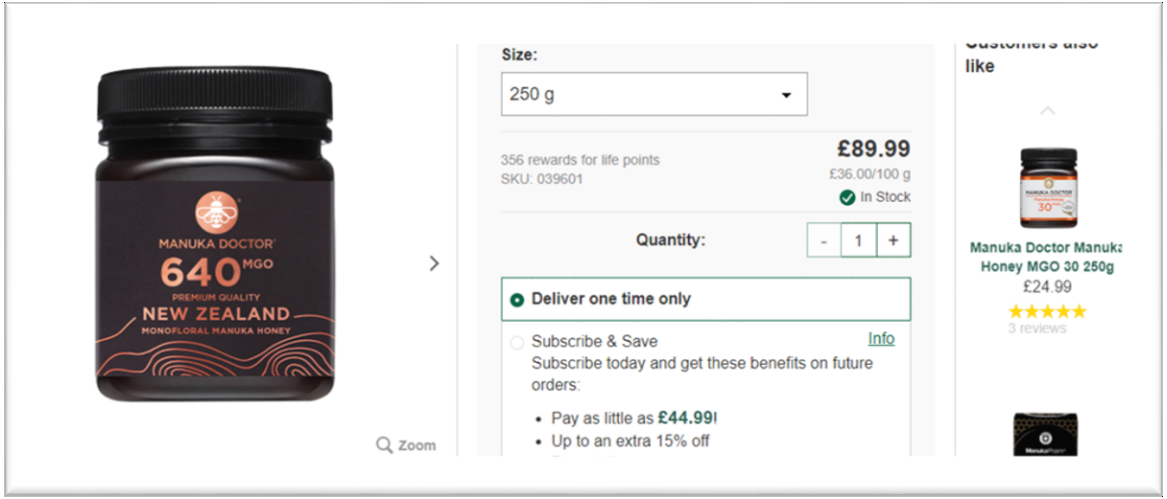
4. Inadequate method of analysis - e.g. morpholine in apples



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, *Food Analytical Methods*, 5(4), 874 - 880

7. Nature springs a surprise

- **7.1 SEM**
- **7.2 Almond/mahaleb**
- **7.3. Mānuka honey SCIRMS**



Random example – not case related…

D. Thorburn Burns, Anne Dillon, John Warren, and Michael J. Walker, 2018, A Critical Review of the Factors Available for the Identification and Determination of Mānuka Honey, *Food Analytical Methods*, 11, 1561 – 1167.

7.1 Semicarbazide (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

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

7.2 Almond or mahaleb – cumin & paprika recalls

ELISA

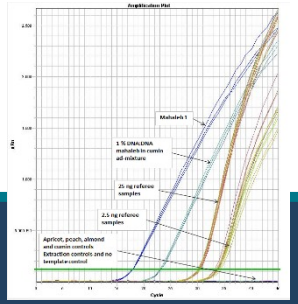
PCR

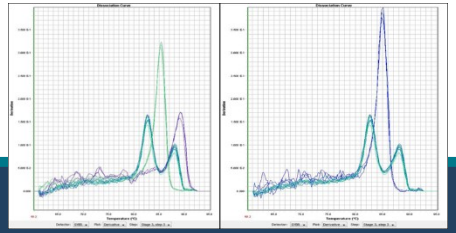
qPCR assay for Mahaleb

PCR screening assay

LC-MS/MS for Prunus Species-specific peptides

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		

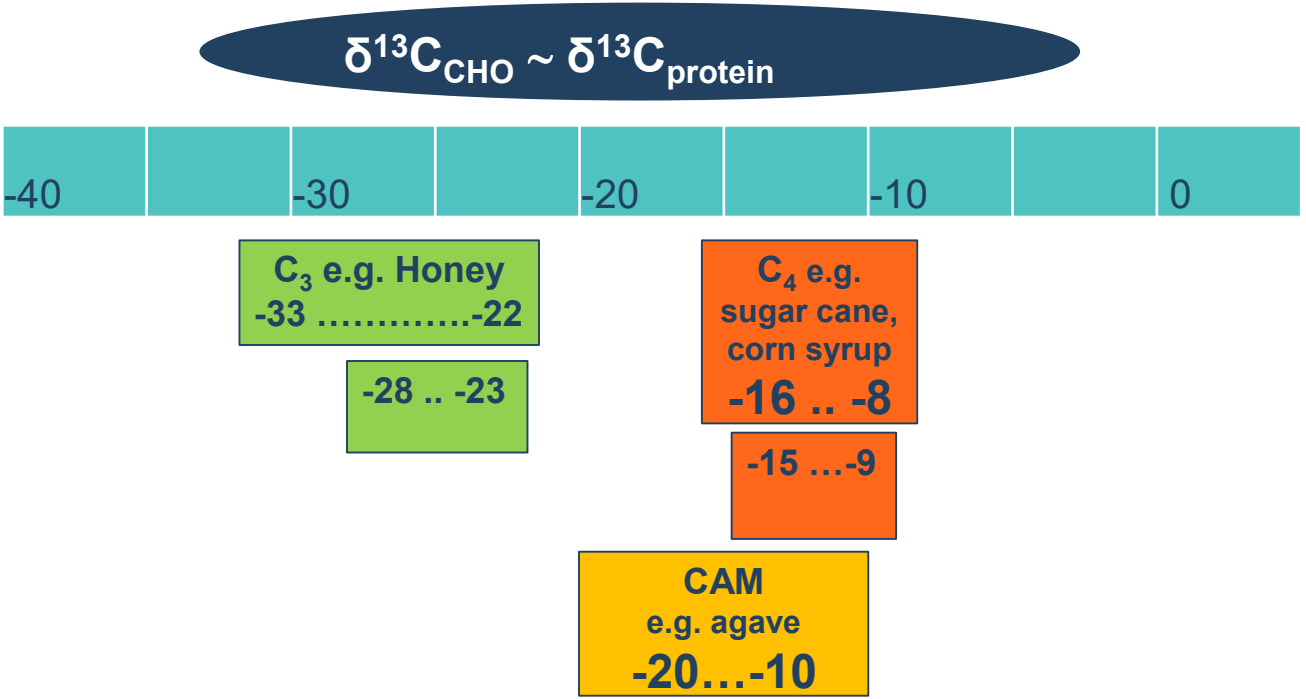




7.2 Almond or mahaleb related publications

1. 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.
2. 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.
3. 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
4. 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.
5. Michael Walker and Malcolm Burns, The Almond and Mahaleb Allergen Story – PCR Resolution of Live Incident Investigations, in: DNA Techniques to Verify Food Authenticity: Applications in Food Fraud, Eds. by Malcolm Burns, Lucy Foster, Michael Walker, Royal Society of Chemistry, London 2019, ISBN 978-1-78801-178-5, pp 154 -161

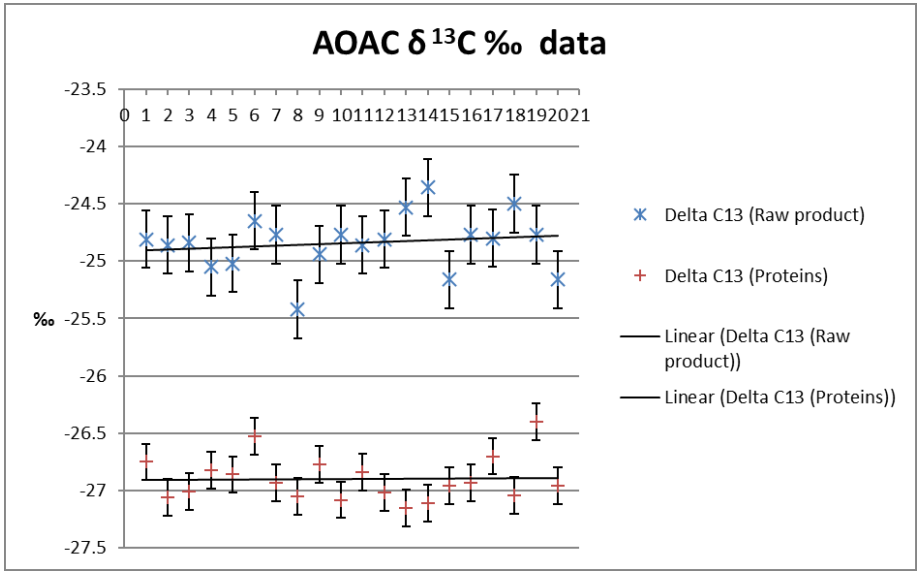
7.3 Mānuka honey – added sugars $\delta^{13}C$ ‰

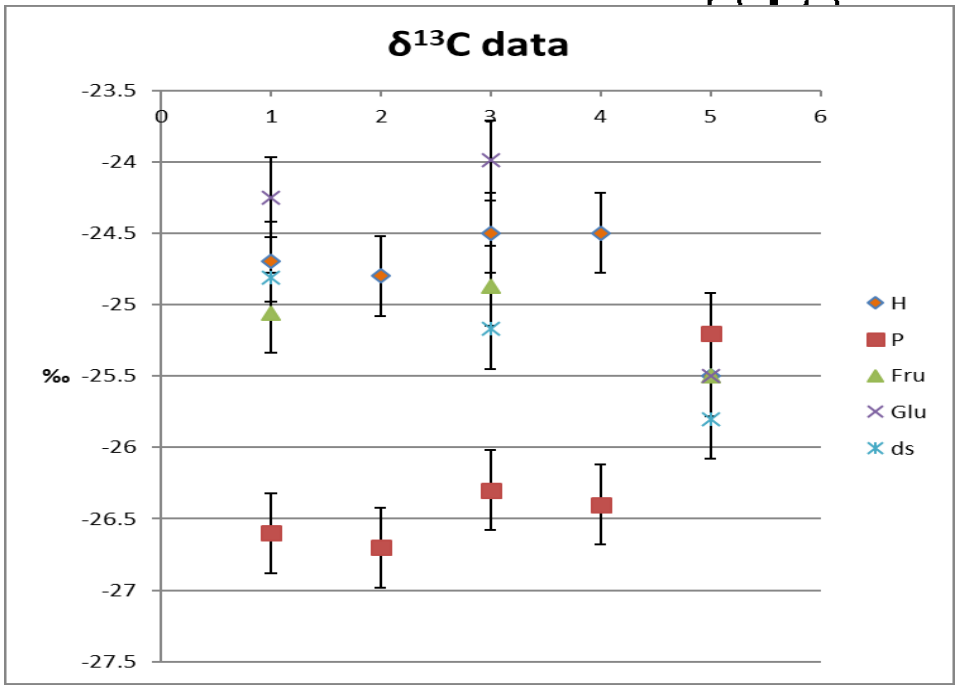


Carter, J.F. and Chesson, L.A. eds., 2017. Food Forensics: Stable Isotopes as a Guide to Authenticity and Origin. CRC Press.

crassulacean acid

7.3 Mānuka honey – added sugars





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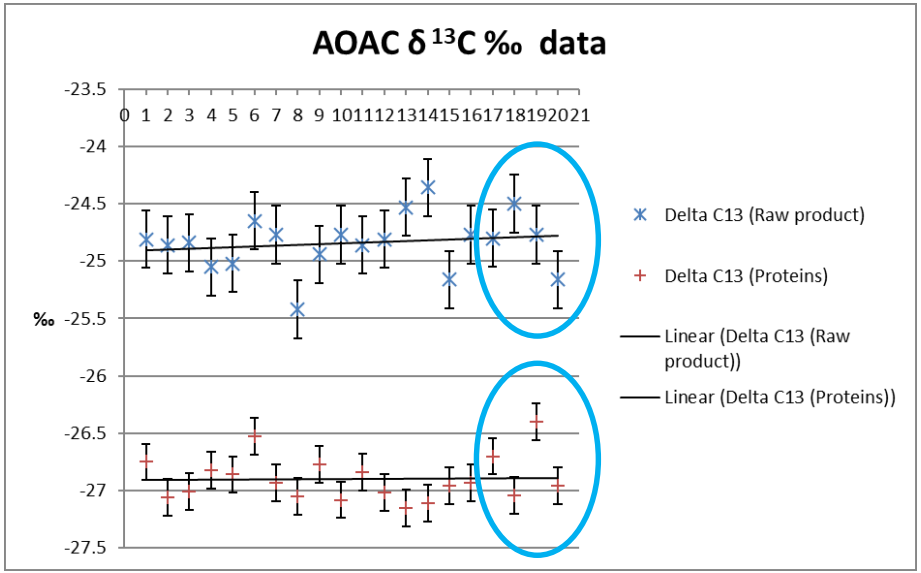

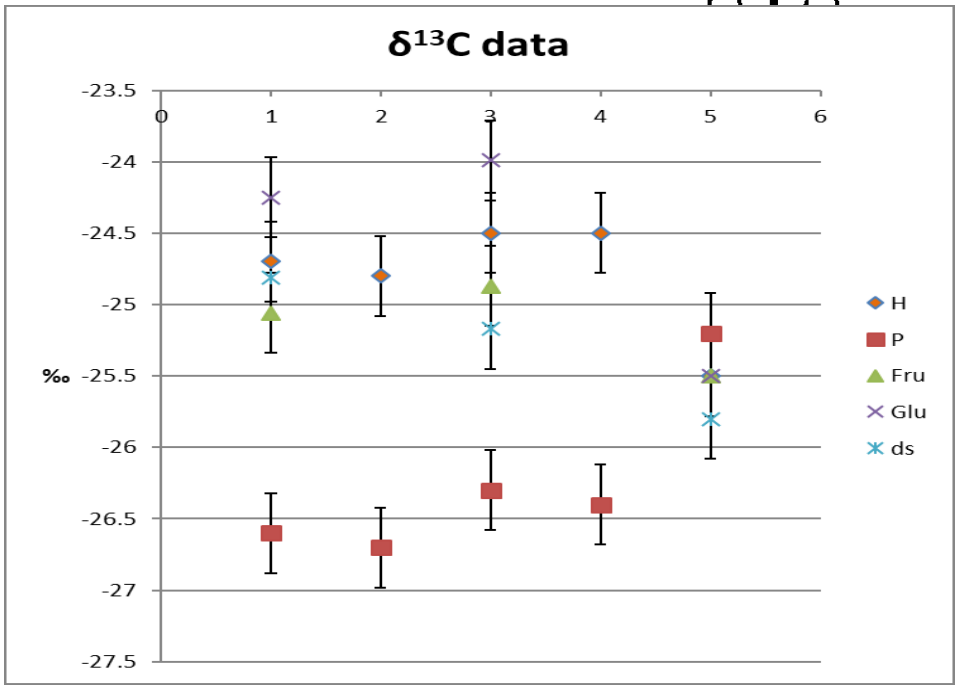

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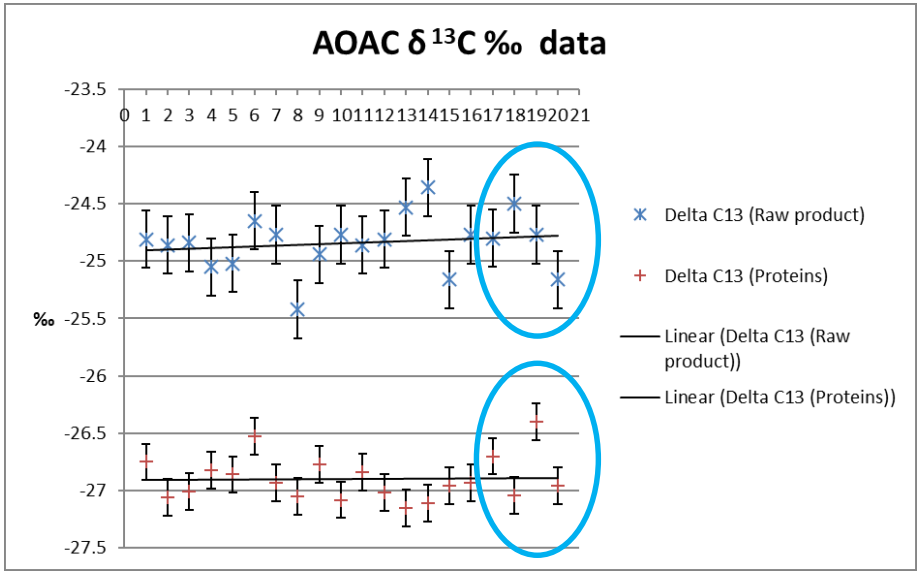
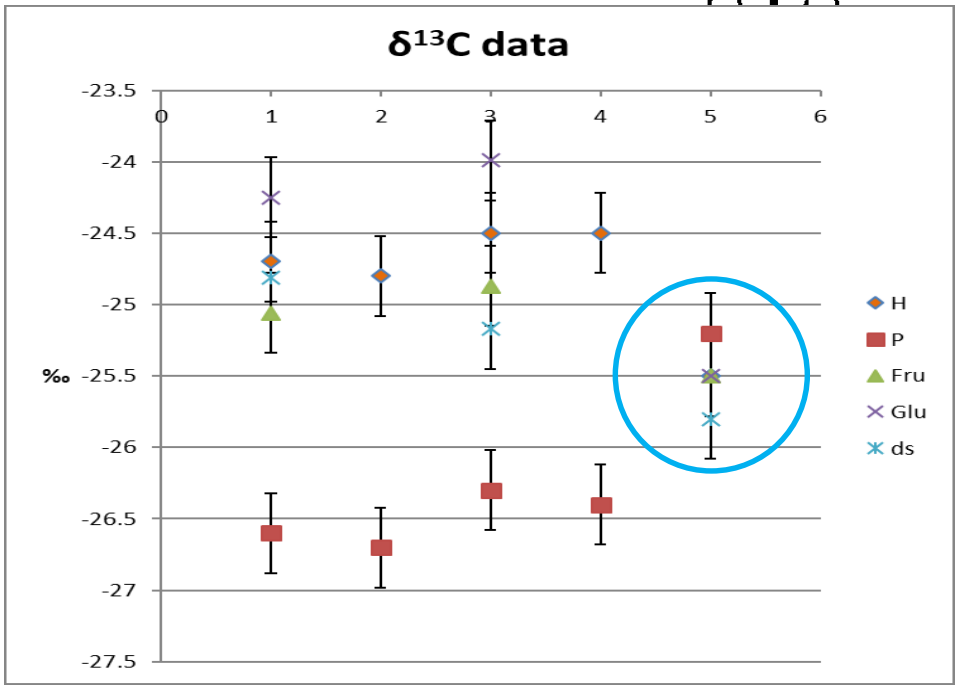




See also …

Contents lists available at ScienceDirect

Science & Justice

journal homepage: www.elsevier.com/locate/scijus

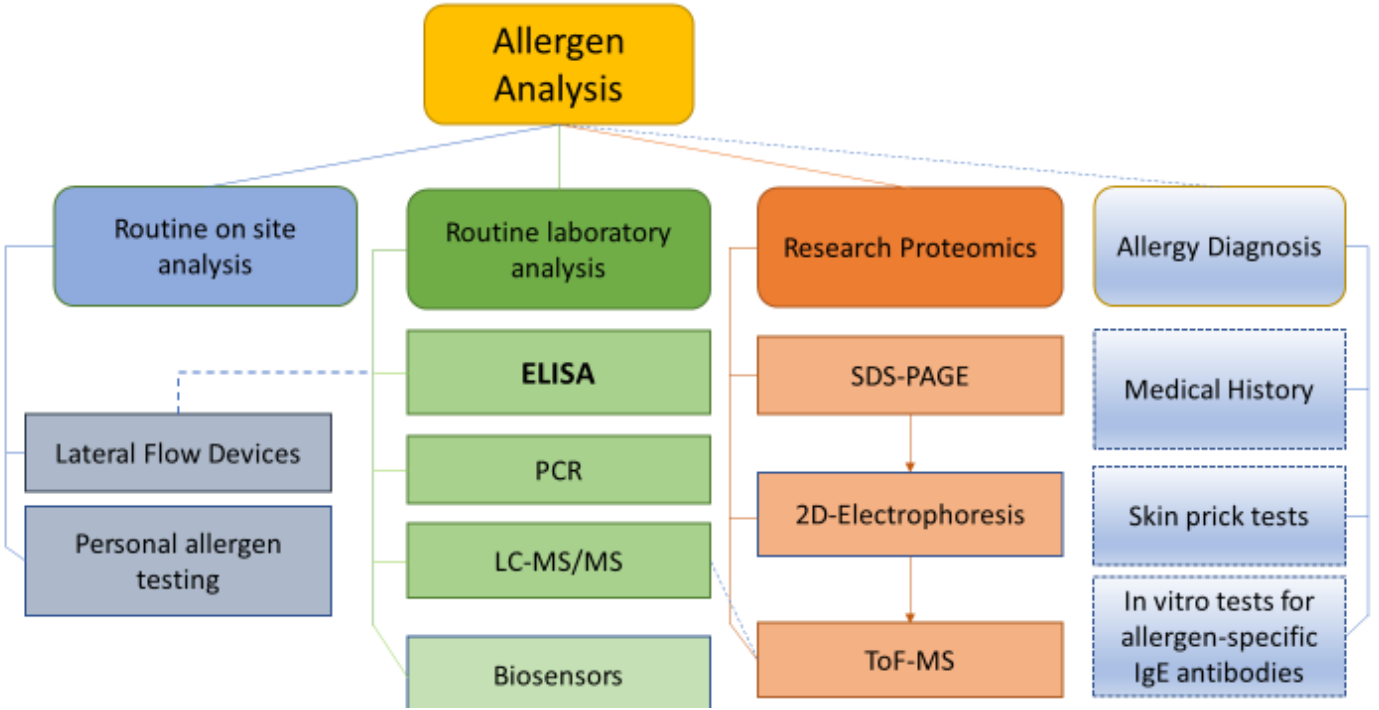
Lessons learned from inter-laboratory studies of carbon isotope analysis of honey

Philip J.H. Dunn[a,*], Sarah Hill[a], Simon Cowen[a], Heidi Goenaga-Infante[a], Mike Sargent[a], Ahmet Ceyhan Gören[b,1], Mine Bilsel[b], Adnan Şimşek[b], Nives Ogrinc[c], Doris Potočnik[c], Paul Armishaw[d], Lu Hai[e], Leonid Konopelko[f], Yan Chubchenko[f], Lesley A. Chesson[g], Gerard van der Peijl[h], Cornelia Blaga[h], Robert Posey[i], Federica Camin[j], Anatoly Chernyshev[k], Sadia A. Chowdhury[l]

Rogers *et al*., 2014. Investigating C-4 sugar contamination of manuka honey and other New Zealand honey varieties using carbon isotopes. J. agric food chem, 62, 2605-2614.

Rogers *et al*., 2014. The unique manuka effect: why New Zealand manuka honey fails the AOAC 998.12 C-4 sugar method. J agric food chemistry, 62, 2615-2622.

Food allergen analysis



Walker, M.J., 2019. Food Allergens: An Update on Analytical Methods. In: Melton, L., Shahidi, F., Varelis, P. (Eds.), Encyclopedia of Food Chemistry, vol. 1, pp. 622–639. Elsevier.,

Food allergen analysis (4) (6)

- Remains problematic but much good work is underway
- In critical situations >1approach if possible
  - e.g. at least 2 ELISA platforms, or ≥2 of ELISA, PCR, LC-MS/MS
- RMs (LGC & MoniQA) – how best to use them!
- When analytical targets differ RMs may not help much
- Bioinformatics of plant allergens still need work
- Reporting - sufficient detail to assist risk assessors and managers
- Upper rather than the lower bound of the MU as datum of interest

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

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.

Allergen QRA webinar

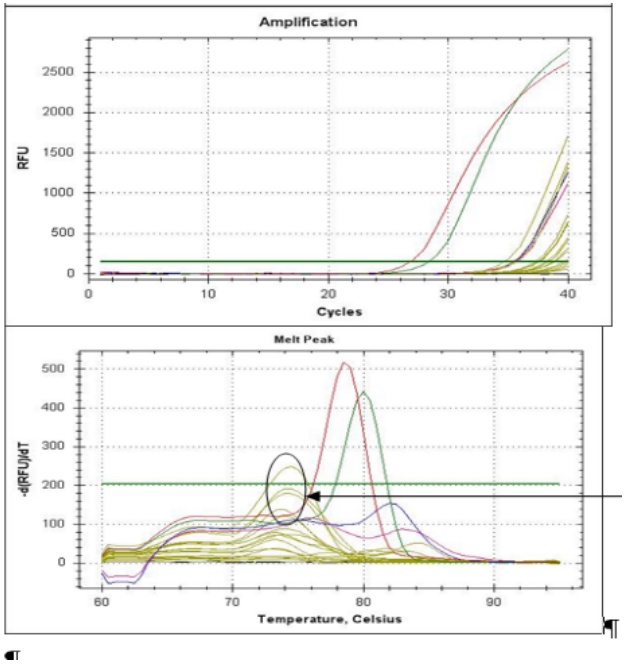
Introduction to the New ILSI Europe Activity on Food Allergen Quantitative Risk Assessment

https://register.gotowebinar.com/register/4360356758752960014

29th June 2020
15 30 – 16 30
UK time

Introduction to the project, providing insight on the aim, importance and outcome of the activity, as well as practical information such as timeline, followed by a short Q&A session.

5. GMO detection - rice



DNA sequences -

- P35S
- TNOS
- CryIAb/CryIAc
- Rice taxon-specific method, e.g. PLD
- SYBR® green assay for Cry1Ab/Ac required

European Reference Laboratory for Genetically Modified Food and Feed, EU-RL GMFF Revised Guidance on the Detection of Genetically Modified Rice Originating from China Using Real-Time PCR for the detection of P-35S, T-nos and Cry1Ab/Ac, version of 2014, ISBN 978-92-79-38478-3.

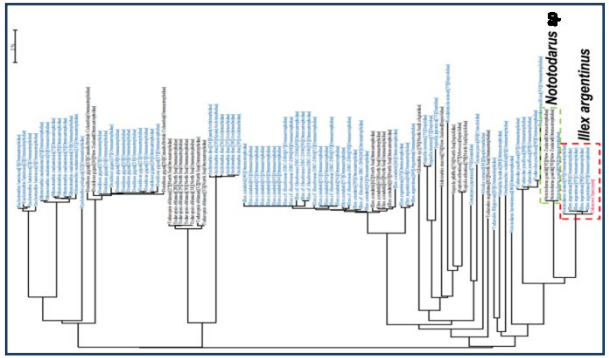
8. Inadequate bioinformatics

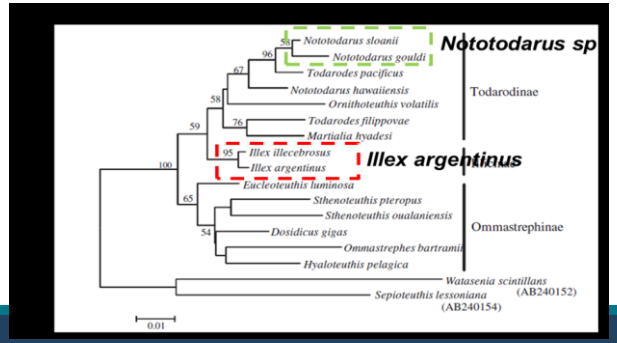
Back label “squid” “Produced in New Zealand and packed in the UK from arrow squid caught in the South West Pacific Ocean ...”

Public Analyst “DNA consistent with that of *Illex argentines”*

Arrow squid - *Nototodarus gouldi* and *Nototodarus sloani*

Differentiation by COI gene data available in '*BOLD*' and 16s rRNA sequence in GenBank



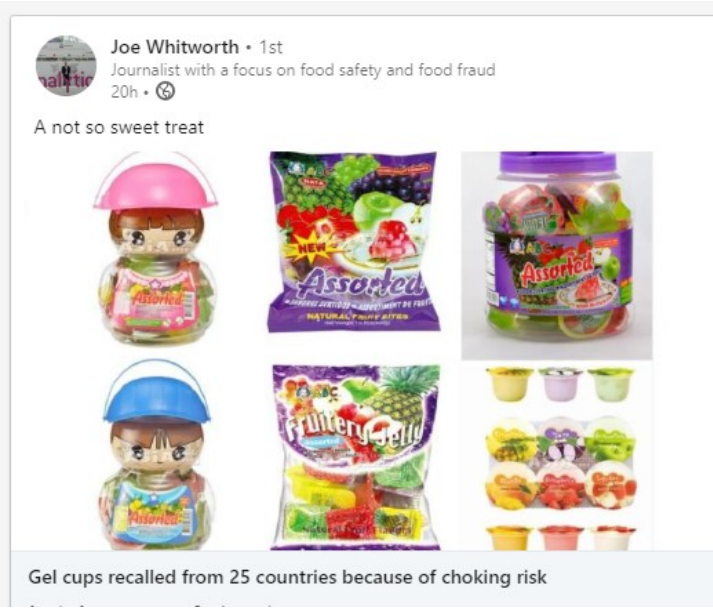


BOLD: Illex and Nototodarus most probable species, > 99% similarity with target sequence

NCBI: 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

Choking hazards (5), (6)



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

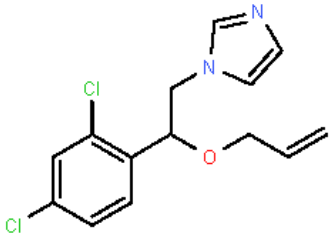
Michael J. Walker, Peter Colwell, Derek Craston, Ian P. Axford & Jack Crane

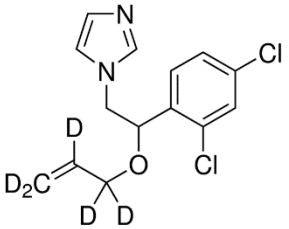
Food Analytical Methods **5**, 54–61(2012) | Cite this article

https://www.gov.uk/government/news/successful-workshop-on-assessment-of-jelly-mini-cups

https://www.linkedin.com/posts/joe-whitworth-30b6b052_gel-cups-recalled-from-25-countries-because-activity-6679719487895298048-YkHr

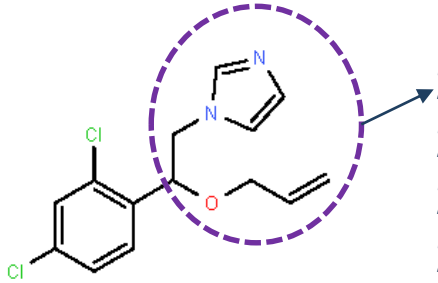
Pesticides – Imazalil (4), (7)





297 → 159 *m/z*
297 → 201 *m/z*
297 → 255 *m/z*
297 → 176 *m/z*
297 → 173 *m/z*
297 → 109 *m/z*

Pesticides - Imazalil



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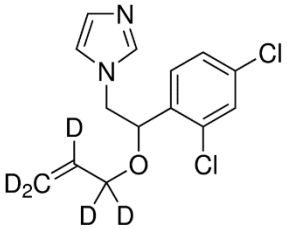
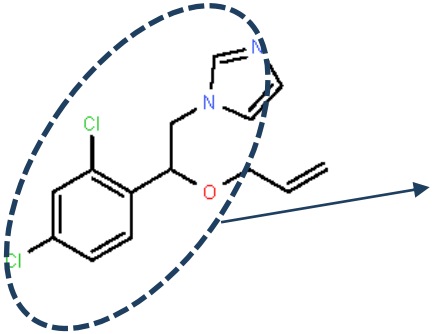


Pesticides - Imazalil



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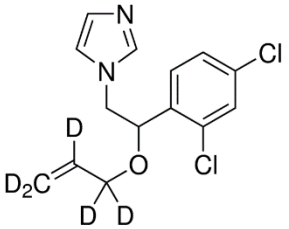

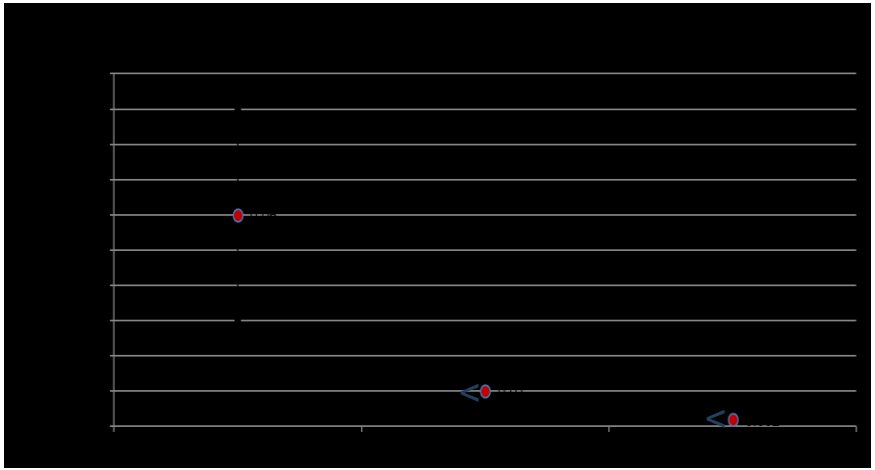

A good laboratory

- ✓ The necessary expertise, equipment and infrastructure
- ✓ Sufficient suitably qualified, trained and experienced staff
- ✓ Performs impartially free from any conflict of interest
- ✓ Delivers in a timely manner, and
- ✓ Accredited and operates to EN ISO/IEC 17025

Reg. 2017/625 *on official controls*, Article 37(4), Designation of official laboratories

Anything else?

- ✓ The necessary expertise, equipment and infrastructure
- ✓ Sufficient suitably qualified, trained and experienced staff
- ✓ Performs impartially free from any conflict of interest
- ✓ Delivers in a timely manner, and
- ✓ Accredited and operates to EN ISO/IEC 17025

Understands the context

- ✓ **Scientific**
- ✓ **Legislative**
- ✓ **Policy**

Reg. 2017/625 *on official controls*, Article 37(4), Designation of official laboratories

Why do laboratories get it wrong?

1. Inadequate planning for sampling - allergens
2. Incorrect sampling - mycotoxins
3. Loss of chain of custody of sample
4. Inadequate method of analysis – morpholine, GMOs, allergens
5. Inadequate application of a method of analysis – choking hazards
6. Inadequate interpretation or reporting – mycotoxins, allergens
7. Nature springs a surprise – SEM, mahaleb, manuka honey SCIRMS
8. Inadequate bioinformatics – squid (but also plant allergens …)


violas
mf

Acknowledgements

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Kirstin Gray
Malcolm Burn
Malvinder Singh
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