

Government Chemist



Chemistry Safeguarding our Food, Quis custodiet ...?

Michael Walker MChemA -- 2015

Science for a safer world





Aims of presentation

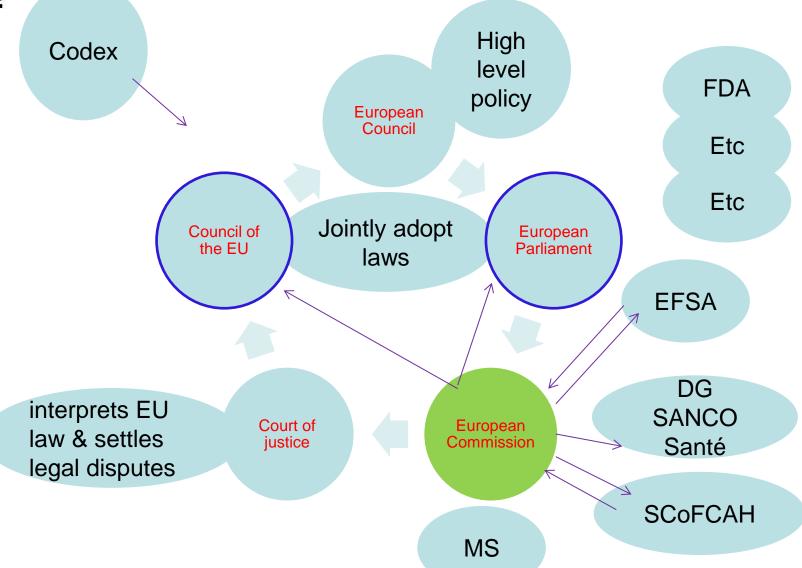
Sed quis custodiet ipsos custodes?*

- European food and feed law
- Technical appeal to the Government Chemist
- Why laboratories get it wrong

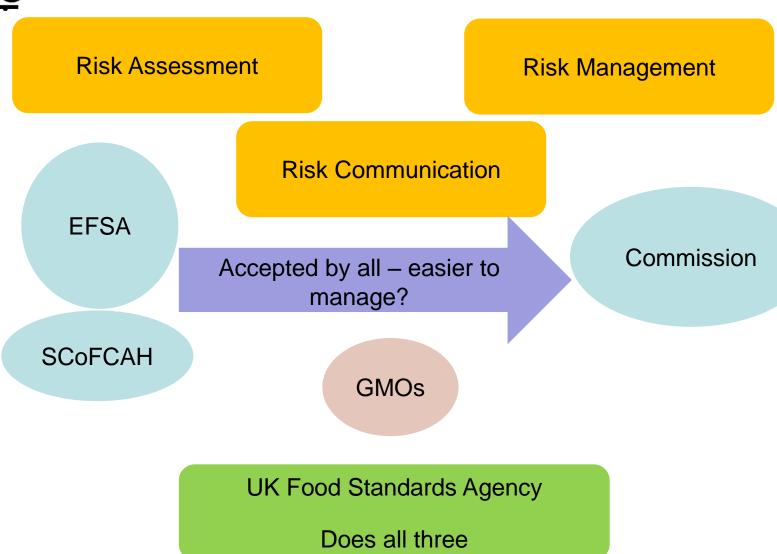
^{*} Decimus Junius Juvenalis, Juvenal, late 1st – early 2nd century AD, Satires, Satura VI, line 347/8



Regulation in the European Union









5 different sorts of 'law'

- Regulations binding in entirety across the EU
- Directives set a goal, individual MS to decide how...
 - Less used now, e.g. food labelling formerly under Directive 2000/13/EC is now controlled by Regulation (EU) No. 1169/2011 came into force 13 December 2014
- Decisions specific & directly binding on those addressed
 - (e.g. any or all MS or an individual company), Decision 2013/287/EU of
 13 June 2013 on unauthorized GM rice
- Recommendations not binding, suggest a line of action
 - Commission Recommendation 2012/154/EU on monitoring of the presence of ergot alkaloids in feed and food
- Opinions not binding, issued by the main EU institutions
- EU legal measures can be accessed at Eur-Lex,

http://eur-lex.europa.eu/homepage.html



What we do - Making it crystal clear

leaks.

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

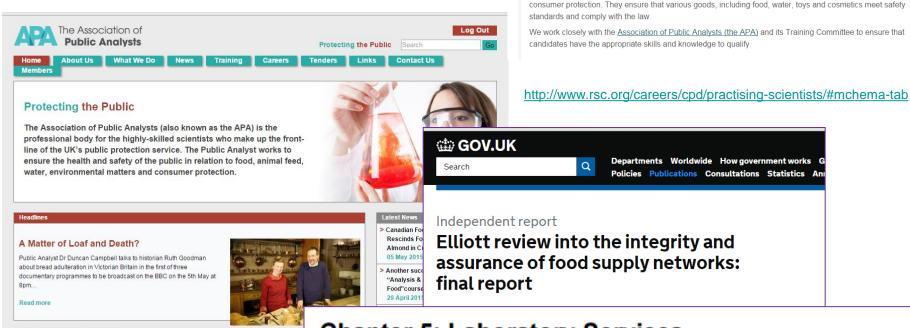
the air we breathe, from checking the safety of children's toys to

providing a frontline emergency response unit for chemical

Enforcement of chemical food safety,

UK, (Rol ...)

Mainly by Public Analysts



Chapter 5: Laboratory Services

Those involved with audit, inspection and enforcement must have access to resilient, sustainable laboratory services that use standardised, tested approaches.

MChemA

We award the Mastership in Chemical Analysis (MChemA) which is the statutory qualification for practice as a

Throughout the UK there is a network of laboratories that specialise in the science of public protection. The Public Analysts work to ensure the health and safety of the public in relation to food standards, environmental matters and

Mastership in Chemical Analysis

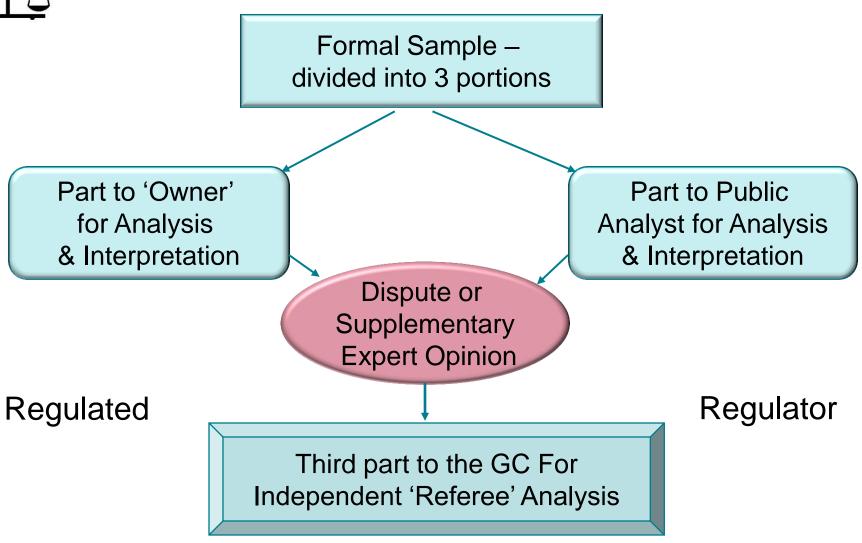
Public Analyst and Agriculture Analyst in the UK

SiLC

Qualified Persons Register



The Referee Process... quis custodiet ...





Cases May 2012 - May 2015

2011/14 Programme

25 cases in 22 months

- 7 Speciation
- 3 pesticides
- 3 Food Contact Materials
- 1 PAH
- 2 Aflatoxins
- 1 Aluminium
- 2 GMO
- 3 Carbon monoxide
- 1 Feed (A, B1, E & Cu)
- 1 added water in fish*
- 1 Origin (farmed / wild)*

2014/17 Programme

22 Cases in 14 months

- 1 Food Allergen
- 2 Food Additives
- 2 Nitrofurans
- 4 Labelling opinion*
- 2 Speciation
- 3 Food Contact Materials
- 4 Aflatoxins
- 1 GMO
- 3 Pesticides

^{*}For some cases advice given no analysis required



Contaminants



Contaminants

- Chemical contaminants are substances not intentionally added to food & may arise as a result of
- Growth, harvest, storage & transport (e.g. mycotoxins)
- Production (e.g. ethyl carbamate)
- Packing (e.g. phthalates)
- Cooking (e.g. acrylamide)
- Or environmental contamination (e.g. Dioxins).
- To prevent risks to human health, the EU has taken measures to regulate the concentrations of certain contaminants in food



Contaminants -- Commission Regulation (EC) No. 1881/2006 sets maxima above which food must not be sold

Type	Contaminant
Mycotoxins	Aflatoxins B ₁ and the sum of B ₂ , G ₁ and G ₂ ; aflatoxin M ₁ in milk; ochratoxin A; patulin; deoxynivalenol; zearalenone; fumonisins; T-2 and HT-2 toxin
Heavy metals	Lead; cadmium; mercury; tin (inorganic)
Agricultural inorganic residues	Nitrates
Chloropropanols	3-Monochloropropane-1,2-diol (3-MCPD)
Complex and aromatic organics	Dioxins and polychlorinated biphenyls (PCBs); polycyclic aromatic hydrocarbons (PAHs)

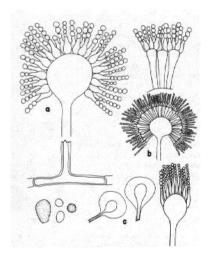
latest version of Reg 1881/2006 should be consulted for up to date limits – see http://eur-lex.europa.eu/homepage.html

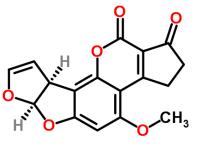


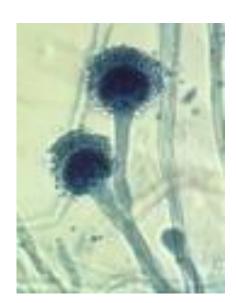
Aflatoxins

- Aflatoxins secondary metabolites of moulds Aspergillus flavus, A. Parasiticus ...genotoxic carcinogens, liver cancer particularly with simultaneous hepatitis B virus infection
- $B_1 B_2 G_1 G_2$
- Aflatoxin M₁ is a major metabolite of aflatoxin B₁ in humans and animals, which may be present in milk from animals fed with aflatoxin B1 contaminated fee
- Analysis ELISA or by extraction, immunoaffinity column clean up followed by liquid chromatography with fluorescence detection and by LC-MS/MS.

http://www.efsa.europa.eu/en/efsajournal/doc/446.pdf
And Chemspider









Sampling & sample preparation

- Methods of sampling and performance criteria for analysis such as recovery and precision are laid down in Commission Regulation No 401/2006.
- Fungal contamination is patchy incremental sampling at import,
- Followed by high shear mixing with a defined amount of water
- Groundnuts (peanuts) for aflatoxins to involve up to 100 incremental sample of about 200 g (typically 100 g for other commodities) depending on the size of the consignment.



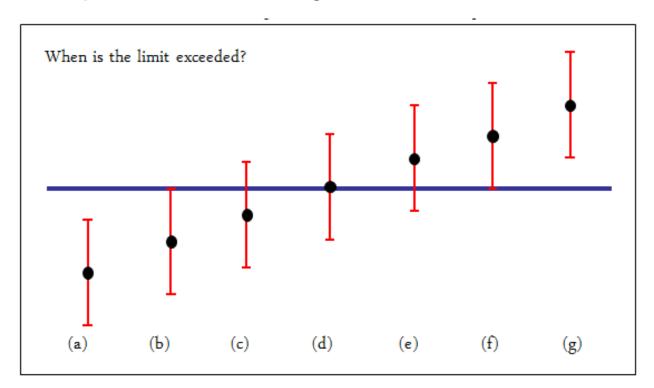


Images courtesy
Kent Scientific Services

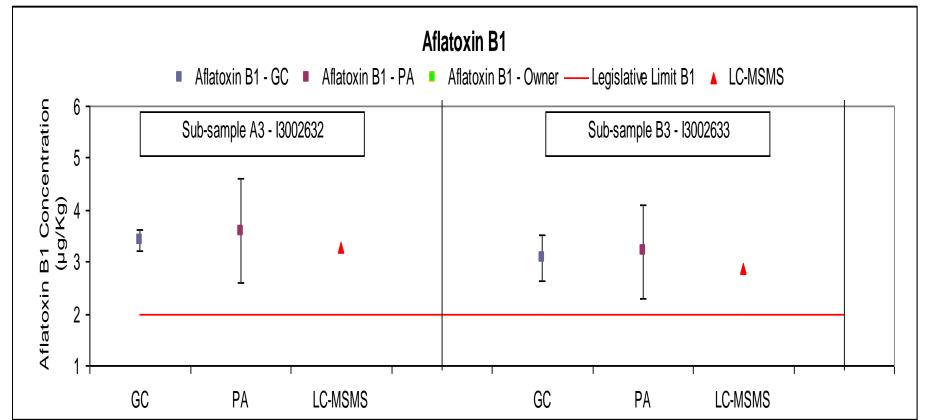


Reporting

- Results are recovery corrected
- Measurement uncertainty calculated as a 95 % CI
- Compliance assessed against x U



Sample	Analysis	Owner Analysis	Dispute	Result & Outcome
Hazelnut Meal	Aflatoxins	No	Supplementary Expert Opinion	Confirmed PA result



Walker et al.,2013, Quis custodiet – a Review of the Resolution of Disputed Chemical Results in the UK Official Feed and Food Control System 2010 – 2011, J Assoc Public Analysts (Online), 41, 1-27

Gray et al., 2014, Effect of Spiking Contact Times on the Analytical Recovery of Aflatoxins, J Assoc Public Analysts (Online),42, 18-34



Heavy Metals – (well, almost...) Aluminium (and Arsenic...)

- The regulated heavy metals Lead; cadmium; mercury; tin (inorganic), are well known and easily dealt with
- Two other elements deserve mention
- Aluminium in Noodles
 - A cause of rejections of consignments of dried noodles at EU border inspection posts
- (Inorganic Arsenic in Rice
 - An issue of current concern in EU)



Dried Noodles - Aluminium

- Problem first noticed in Started in Germany 2008 findings of 50 to 150 mg kg⁻¹ Al in dried noodles
- TWI for AI -- 1 mg/kg bw/week (EFSA) precautionary ... animal studies that suggested the potential for neurotoxicity, embryotoxicity, male reproductive system and developing nervous system effects
- TWI was reviewed again in 2011 by JECFA (2011) which established a provisional tolerable weekly intake (PTWI) of 2 mg/kg body weight
- TWI may be exceeded in some of the UK population
- Most food contains less than 5 mg kg⁻¹ of aluminium
- "Limit" stemmed from a view taken by the European Commission supported by the Standing Committee on the Food Chain and Animal Health



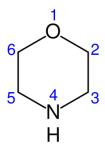
- The relevant minute of SCoFCAH
- "Following requests for clarification as regards the proposed listing of noodles from China under Annex I to Regulation (EC) No 669/2009, the Commission clarified that, based upon available data, the level of 10 mg/kg could be used to distinguish noodles with acceptable unavoidable background presence of aluminium from noodles presenting unacceptable levels. Vote: qualified majority by 338 votes in favour, 7 votes abstained."
- it would be interesting to investigate the natural occurrence of aluminium in Asian wheat flour.



Food Additives



Morpholine in apples



- Cyclic secondary amine ether, used as a carrier/emulsifier for glazing agents applied to fruit & permitted in various countries, e.g. Chile, the USA and Canada
- Morpholine is a precursor of carcinogenic nitrosamines although the possibility of their formation in the human stomach after ingestion of morpholine-treated apples is reported as highly unlikely
- No application to approve in EU, therefore it should not be present in food on sale in member states.



dansyl chloride

(dimethylamino)naphthalene-1-sulfonyl chloride

$$\begin{pmatrix} H \\ N \\ O \end{pmatrix}$$

$$H_3C-N$$
 CH_3
 O
 S
 N
 N
 N
 N

H₃C

H₃C

dabsyl chloride

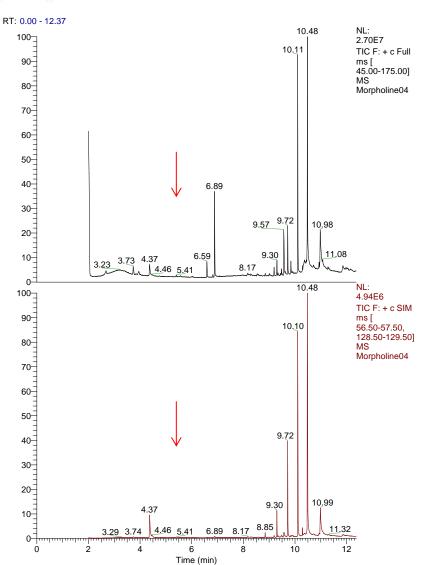
4-(dimethylamino)azobenzene-4'-sulfonyl chloride

H₃C

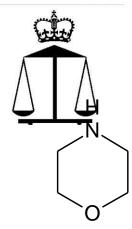
H₃C



Amine acetylation derivatisation - which fragment ions From morpholine group detected



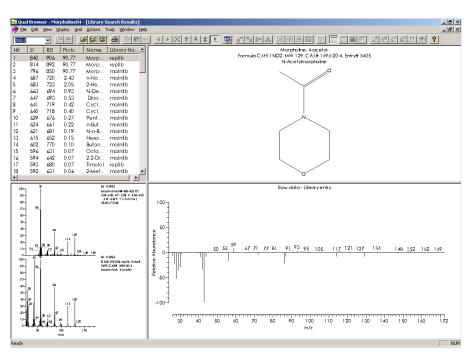
GC/MS full scan TIC (upper) and SIM (lower) of acetylated morpholine spiked into a matrix sample at 2 mg kg⁻¹. The retention time of the morpholine derivative is 4.37 minutes.

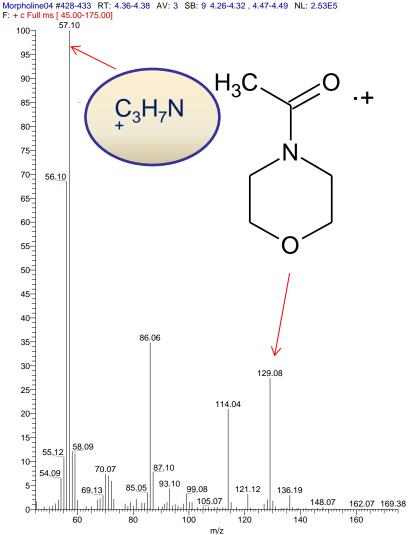


GC-MS

Acetic anhydride

H₃C O

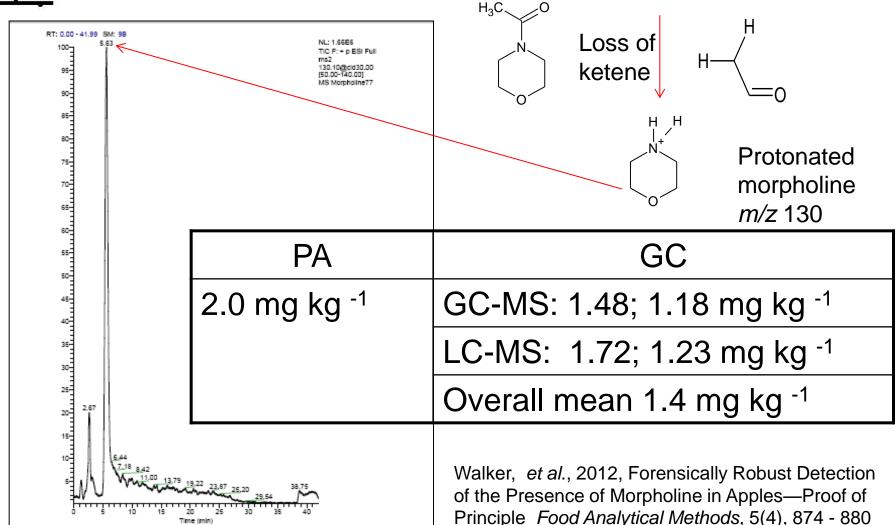






LC-MS

Fig. 2, LC/MS chromatogram of the productions of protonated acetylated morpholine spiked into a matrix sample at 2ppm (m/z=130). The retention time of the morpholine derivative is 5.63



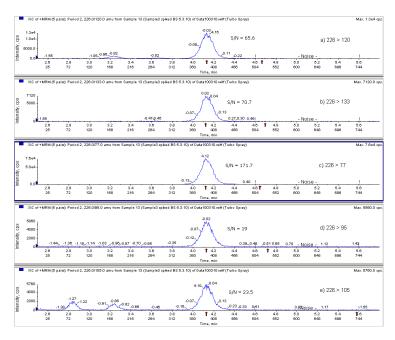


Illegal dyes Dimethyl Yellow in Turmeric

Sample - 15% turmeric oleoresin, 85% emulsifier, 'Crestasorb 80' believed to be polysorbate 80. Turmeric oleoresins produced by extraction of root turmeric powder by solvents - extracts contain natural pigments, curcumins, together with turmeric oil, a complex mixture containing tumerones, sesquiterpenes, zetenes, phellandrene and cumene



Method



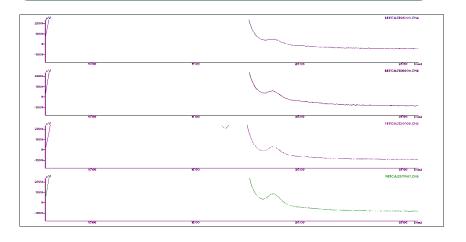
Walker et al. 2012 Forensically Robust Determination of the Illegal Dye Dimethyl Yellow in a Refractory Curcuma Oleoresin–Surfactant Matrix a Case Study, Food Analytical Methods, 6, 395-405

Extract and partition Hexane/MeCN/MeOH/H₂O:NaCl

Hexane to dryness, dissolve in cyclohexane/EtAc

GPC

LC-MS





Carbon monoxide



Non-approved food additive

in fish





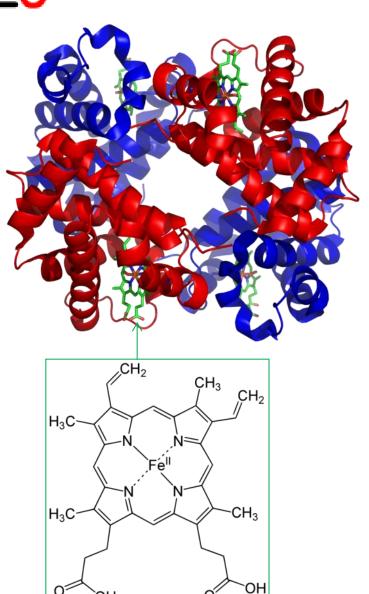
Carbon monoxide C≡o

Haemoglobin / myoglobin CO-haem adduct is cherry red



Apparently fresh appearance
But histamine production continues

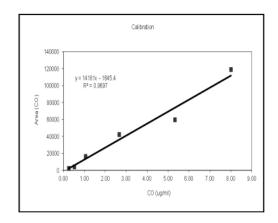
CO is not a permitted food additive





Analysis and issues

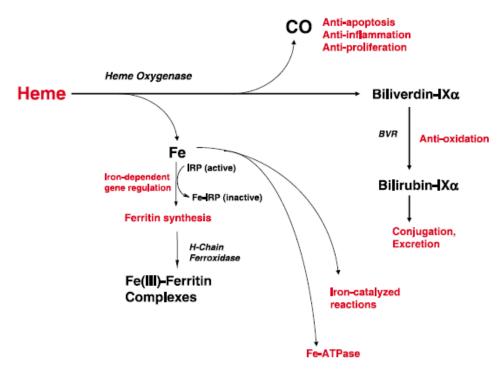
- Headspace GC-MS m/z 28
- Molecular sieve column
- PV = nRT



CR Anderson and Wen-Hsin Wu, 2005, J. Agric. Food Chem., 53: 7019–7023

Naturally occurring?

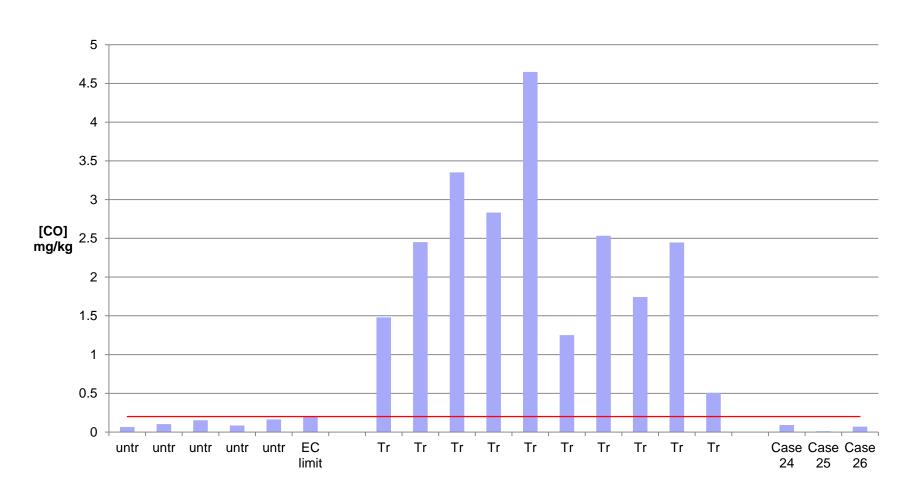
HEME OXYGENASE/CARBON MONOXIDE



Ryter et al., 2006, Physiol Rev 86:583-650

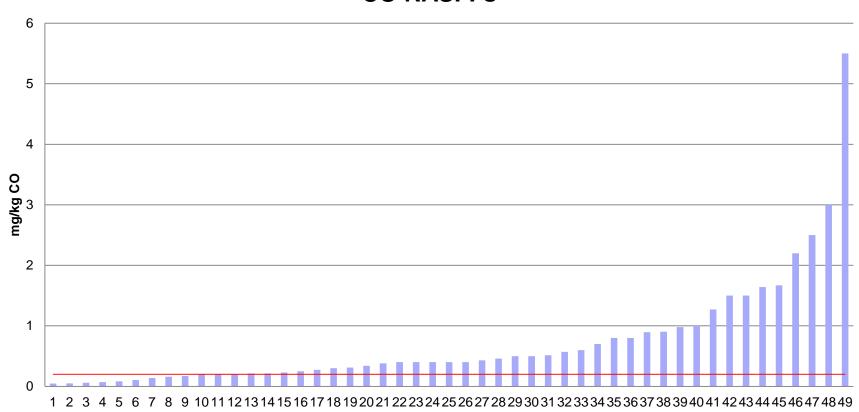


Comparison untreated and treated fish





CO RASFFs





Veterinary drug residues



Nitrofurans

$$O_2N$$
 O NH NH_2

Background

- Veterinary antibiotics widely used prior to ban
- Carcinogenicity
- Parent drugs t_{1/2} v short
- Metabolites used as marker residues
- Annex IV 2377/90 lists cpds for which no MRL can be set – nitrofurans included in this annex – zero tolerance in food producing animals

Analysis

- ELISAs (FoodBRAND project in QUB)
- Acid hydrolysis
- nitrophenyl derivatives LC-MS/MS, isotopically labeled standards
- Minimum required performance limit, MRPL 1.0 µg kg⁻¹
- CCa
- CCβ





Nitrofuran marker metabolites

Parent compound	Metabolite (residue)
Furazolidone	3-amino-oxazolidinone (AOZ)
Furaltadone	3-amino-5-morpholinomethyl-1,3-oxazolidinone (AMOZ)
Nitrofurantoine	1-aminohydantoin (AHD)
Nitrofurazone	Semicarbazide (SEM).

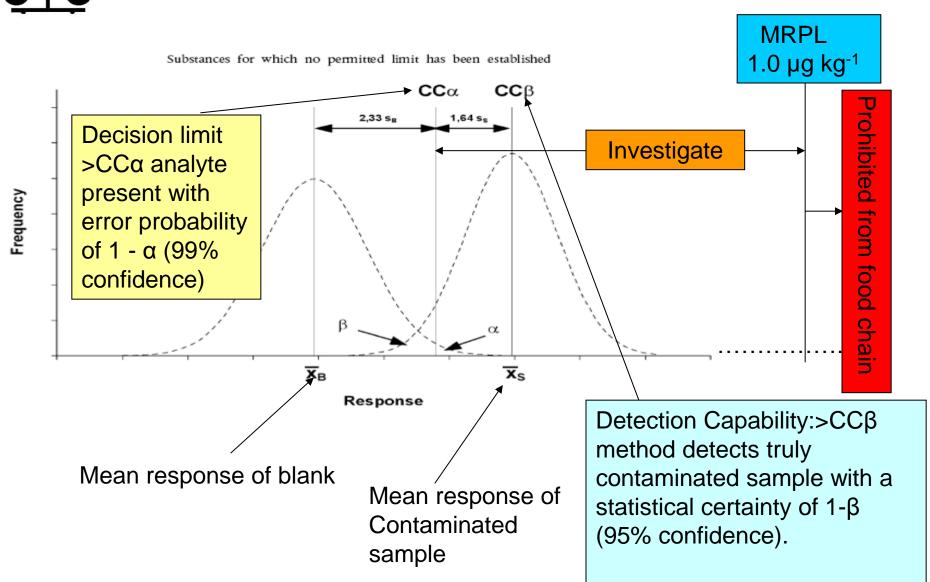


Determination of Semicarbazide metabolite

Source, structures: Vass *et al.* 2008, Nitrofuran Antibiotics: A Review....Veterinarni Medicina, 53, 469 500

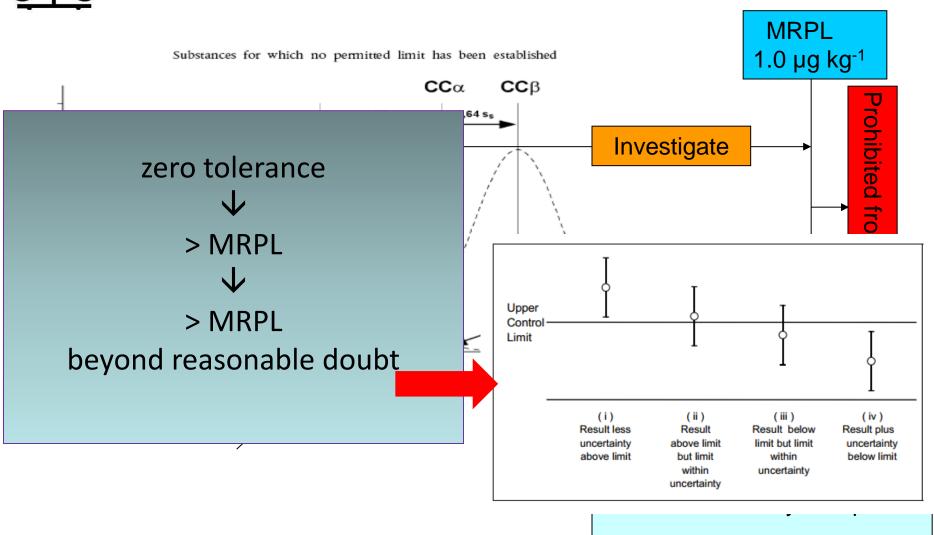


What does "zero" mean - Commission Decision 2002/657/EC amended by 2003/181/EC -





Commission Decision 2002/657/EC amended by 2003/181/EC





SEM formation in shell

SEM in MACROBRACHIUM ROSENBERGII

 Sample analyzed in Belgium and positive on SEM

Whole prawn: 7 ppb

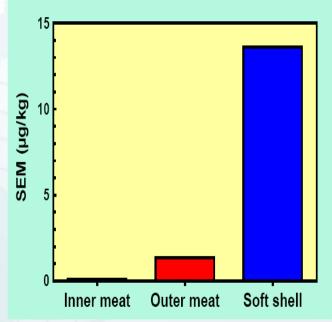
Meat: compliant

Shell: 25 ppb



Van Poucke C, et al., Investigation into the possible natural occurence of semicarbazide in Macrobrachium rosenbergii prawns.

J Agric Food Chem. 2011 Mar 9;59(5):2107-12.
Epub 2011 Feb 7.





The occurrence of semicarbazide in the meat and shell of Bangladeshi fresh-water shrimp.

Glenn Kennedy

Head of Chemical Surveillance Branch AFBI-Stormont Belfast, Northern Ireland, UK



SEM – Sample preparation





- Shells were removed
- Central core flesh excised
- Core and outer flesh separately homogenised for analysis
- CONFIRMED CORE FLESH COMPLIANT





Marker metabolites

Parent drug	Marker	Comments
Furazolidone	AOZ	No other sources or natural
Furaltadone	AMOZ	formation to the best of our knowledge
Nitrofurantoine	AHD	
Nitrofurazone	SEM	Sources other than nitrofurazone and is naturally produced in crustacean shells



Nitrofurans - issues

Case		PA	FBO	GC			
3	AOZ	11.0	< 0.05	<0.05 (3.5)			
6	AOZ	2.0	SEO	32.4			

- Sampling
- What to analyse drained sample or whole thing
- Application of MU
- SEM Core flesh
- Toxicology

- Alternative marker to SEM for Nitrofurazone
 - JRC project, J.A. van Rhijn *et al.*,
 2006, RIKILT cyano-metabolite
 - Wang et al., 2010, Cyano
 Metabolite, J. Agric. Food Chem.,
 58, 313–316

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





Molecular Biology - Speciation

Horse meat and more





Speciation

*(other than beef ... except squid case)

**expressed w/w as gravimetrically prepared raw lean meat species in beef

	Public Analyst*	FBO	GC			
2013	Horse 1 – 5 %	Not seen	Horse ≥ equivalent of 1 %**			
2013	Pork 5 – 30 % (Sheep 30 – 60 %)	Not seen	Pork ≥ equivalent of 0.5 % and possibly ≥ than 1 % **			
2013	Pork 1 – 5 %	Not seen	Approx equivalent to 0.5 % > Pork > 0.1 % **			
2013	Not seen said to have reported > 1 % horse	Not seen	Horse approx equivalent of 0.1 % **			
2013	Horse 1 – 5 %	Not seen	Horse ≥ equivalent of 1 %**			
2013	Beef cheek meat lamb present	Lamb not detected	Confirmed lamb not detected			
2013	Horse 1 – 5 %	Horse ND 1 % detected 0.1 %	Horse ~ equivalent of 1 %**			
2014	Horse	Sample lost	See below			
2014	Squid		Bioinformatics deficit			



Follow up - all open access

Journal of the Association of Public Analysts (Online) 2013 41 67-106 Walker et al

Horse Meat in Beef Products-Species Substitution 2013

Michael J Walker^a, Malcolm Burns^b and D Thorburn Burns^c

Carry over study

Elahi FA0137 A project to establish whether carry-over of meat species occurs in UK meat processing plants during the GMP production of mince meat

Journal of the Association of Public Analysts (Online) 2014 **42 001-017**Busby and Burns

Method Verification of the LOD Associated with PCR Approaches for the Detection of Horse Meat

Eloise Busby^a and Malcolm Burns^{a,b}



2014 Alleged horse meat case

- GC approached in September 2014 when case was about to go to court
- Public Analyst reported DNA:
 - 50 % Pork,
 - 46 % Equine,
 - 4 % Beef,
 - 0.1 % Chicken
- Trader requested referee analysis but own sample lost hence no formal dispute
- To safeguard public funds we ask for evidence of a dispute





Alternatives offered to parties involved

- Ask the court to refer the retained portion
- Offer SEO at full cost
- If FBO can show effort made to have his portion analysed accept the retained portion, homogenise it and forward half to a lab of the FBO's choice for analysis at his expense









Enfield Expo Foods fined over horsemeat sausage



Managing director Ozgur Yilmaz declined to comment after leaving court

A food import company has been fined £5,000 after sausagemeat that was supposed to be pork was found to contain nearly 50% horsemeat.

FBO's lab found 29 % horse



Eight pillars of food integrity supporting improving the UK food supply chain



Consumers first
Zero tolerance
Intelligence gathering
Laboratory services
Audit
Government support
Leadership
Crisis management



Elliott Review into the Integrity and Assurance of Food Supply Networks – Final Report

A National Food Crime Prevention Framework

July 2014





Molecular Biology - GMOs

Rice & rice products from China





GMO - Rice from China -

- No GM rice products authorized in the EU
- 2006 rice products from China contaminated by GMO Bt63
- Commission Implementing Decision 2011/884/EU
- Analytical report and health certificate for each lot
- Consignments sampled at the point of entry into the EU
- Real-time PCR for at least genetic elements (DNA sequences characteristic for):
 - the 35S promoter derived from Cauliflower Mosaic Virus (P35S)
 - the nopaline synthase terminator (TNOS) derived from Agrobacterium tumefaciens
 - the genetically engineered CryIAb/CryIAc

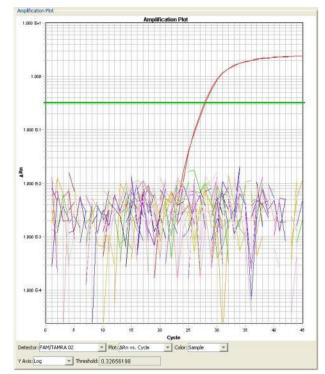


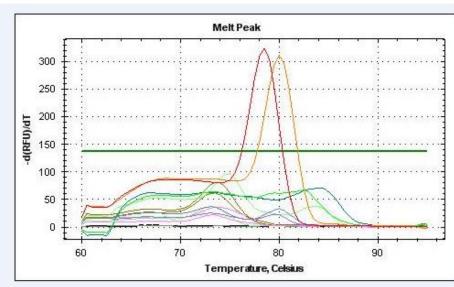
Screening methods

Specific fluorescent probe (Taqman) or DNA binding (intercalating) fluorescent dyes (e.g., SYBR Green)

SYBR Green binds to DNA \rightarrow C_t But may also bind to nonspecific PCR products and primer dimers

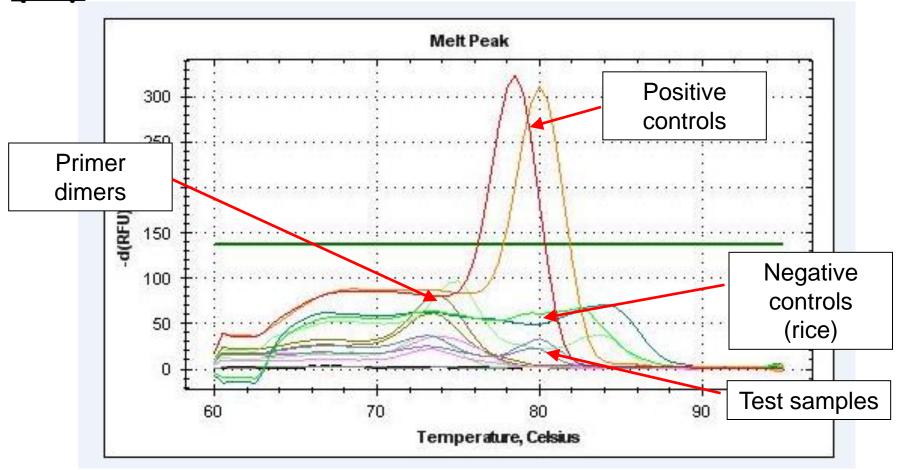
Derivative of fluorescence v temperature plotted \rightarrow DNA fragment melting point T_m







Melt curve (first derivative)







	DNA	BioRad				ABI 7900			
	Extract	Plate 1		Plate 2		Plate 1		Plate 2	
		Ct	Tm	Ct	Tm	Ct	Tm	Ct	Tm
	Bt11	+	+	+	+	+	+	+	+
	Mon810	+	+	+	+	+	+	+	+
	w/t rice	-	•	-	•	-	•	-	•
	LLRICE62	+	+	+	+	+	+	+	+
	E1	+	*	-	i	+	+	-	-
	E2	_	-	-	-	+	+	_	+
	E3	-	ı	+	*	_	ı	-	i
	E4	_	-	+	+*		-	_	-
	E5	_	-	+	-	_	+	_	-
	E6	-	•	+	+*	_	•	+	+
	E 7	+	+*	+	+	-	-	-	-
	E8	_	-	+	+*	+	+	_	+
	H1	-	-		•	-	-	-	•
	H2	-	-	+	+*	+	+	-	-
	H3	+	*	-	ı	_	ı	-	i
	H4	-	ı		ı	-	ı	-	I
	H5	_	-	_	_		-	_	-
	H6	_	-	-	-	_	-	_	-
	H7	-	-	_	-	_	-	_	-
	H8	-	-	_	-		-	-	+
	NTC	-	-	_	-	-	-	-	_



Seminar

24th June, a few days after revised guidance was issued by the European Commission

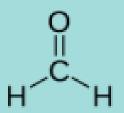


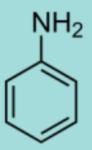




Interactive workshop for detection of Chinese GM rice







LGC

Formaldehyde, Aniline (PAA)



Food Contact Materials Melamine and nylon

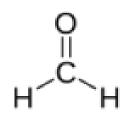








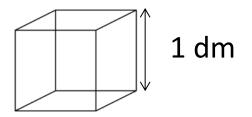
Analysis and Issues



Migration into 3 % v/v aqueous acetic acid 70°C

Surface area/volume ratio

Conventional ratio

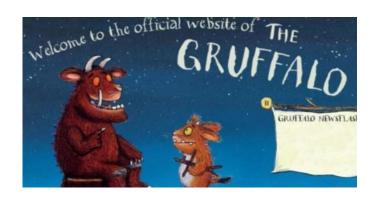


- Regulation (EU) No 10/2011
- Limit 15 mg kg⁻¹
- Expressed on?
- Small objects higher surface to volume ratio
- Normalise to conventional ratio
- But infants & small children higher food intake per kg bw & underdeveloped nutrition & physiology don't normalise
- Multiple items some comply others not ⇒ GMP



Is it intended for infants or children?

- Infant up to 12 months, young child up to 36 months
- "Recommended age range 6
 months +", "Always check food
 temperature before feeding" and
 "always use this product with
 adult supervision"
- 'The Gruffalo' Julia Donaldson & Axel Scheffler, 1990
- "Recommended for children from 36 months"

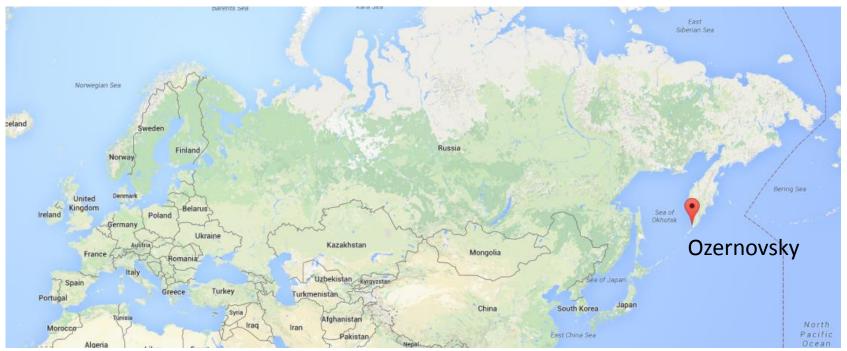






Food authenticity – farmed or wild

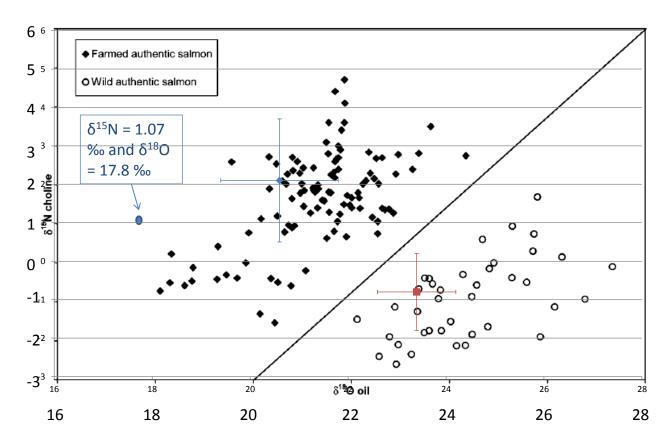




Public Analyst applied

- \triangleright Elemental analysis-isotope ratio mass spectrometry (EA-IRMS) for the δ^{18} O isotope and the δ^{13} C in the fish lipids and the δ^{15} N in choline, a quaternary ammonium essential nutrient for fish
- Lipid fatty acid profile
- ➤ Concluded that the isotopic parameters of the sample were not typical of wild salmon. All results given





FA profile (Public Analyst) C18:2n-6 was not detected; around 4.4 \pm 2.1 % would have been expected if the fish had been farmed

- ➤ More extensive datasets for authentic salmon
- Publish existing results
- Decision tree approach e.g.:
- Further analysis e.g. is the species correct by DNA
- > Another technique



Conclusions – why do labs get it

wrong?

- Sampling
- Differences in methods / Sub-optimal methods
- Chemistry / molecular biology overlooked
- Stochastic ...
- Lack of awareness of context

- Limits
- Appropriate calculation of results
- Application of MU ... statistics
- Population sub-group exposed
- Interpretation of 'black box' algorithms
- Naturally occurring
- Presentation of results
- Adequate datasets covering natural variation



Take home messages

- Confirm 80 % 85 % of OCL findings
- Converse
- Sound measurement science required yes
- But more purchasers of analytical services should demand not only technical competence but also informed interpretation
- Government Chemist in a small subset of cases is a backstop against unwitting errors in analysis and interpretation
- Sustainable repository of experience and skills routinely available



Referee cases UK Government Chemist





Government Chemist

Submit a referee sample to the Government Chemist Submit a supplementary expert opinion sample

Part of National Measurement Office



5 January 2015 - News story

2014 Government Chemist conference report and presentations

Experts outline how science, regulation and integrity within the food industry can combat food fraud during a 2-day conference in November.

https://www.gov.uk/government/organisations/government-chemist



Further reading

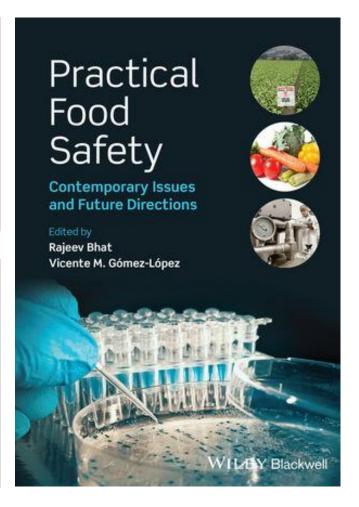
Achieving Quality Chemical Measurements in Foods

Yiu-chung Wong¹ and Michael Walker²
¹Government Laboratory, Homantin Government Offices, Hong Kong, China

²Laboratory of the Government Chemist, Teddington, Middlesex, UK

Protection of the Agri-Food Chain by Chemical Analysis: The European Context

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