





“ Experienced and dedicated staff are the cornerstone of every referee case ”



## Contents

Foreword	2
Section	
1 Remit	4
2 Science underpinning sound dispute resolution	8
3 Impact	13
Publications	25
Glossary	26



# Foreword



This annual review of the activities of the Government Chemist covers a year where public confidence in the UK food chain came under a particular spotlight. The fallout of the contamination of beef with horse meat, which was reported in January 2013, continues to unfold. Government Chemist staff played an important role in providing scientific advice and rigour around the testing regimes used to identify and semi-quantify the levels of adulteration of meat products; and, related disputes provided a high case load for our Referee Function. This work is described in some detail within this review.

Sound measurement science plays a critical role in ensuring the safety and authenticity of food and animal feed. The complexity of supply chains and the advances in products and manufacturing practices provide increasing challenges for the measurement community in building confidence in the marketplace. The Government Chemist Function requires developing and validating methods for existing and emerging analytical challenges and supports both industry and the regulator in providing a source of technical review to resolve discrepancies between differing analytical data. The resolution of disputes is described in section 2 of this review with more detailed description of cases in carbon monoxide treatment of fish, chemical release from food ware and trace contamination. In looking to the future, section 3 covers science development in areas of emerging concern including food allergens, nanoparticles and metal speciation.

It is important that the learning from Referee work and capability building is shared with all relevant stakeholders. I am pleased,

therefore, that we have been able to disseminate our work this year through a range of media, and I would particularly encourage you to visit our web site ([www.governmentchemist.org.uk](http://www.governmentchemist.org.uk)) and to follow our blog (<http://governmentchemist.wordpress.com/>). We also ran our first Webinar this year on the subject of allergen management in the food industry

The work described within this report is the culmination of the efforts of a dedicated and knowledgeable team (page 8) of scientists that deliver the analysis, interpretation and dissemination activities. It is not usual in my foreword to single out particular contributions, but given the magnitude of effort required early in the year I would like to pay particular tribute to Michael Walker, Malcolm Burns, Selvarani Elahi and Kirstin Gray for their efforts on meat authenticity, and to Steve Wood for his overall management of our programmes. I would also like to thank the Government Chemist Working Group and the National Measurement Office for their support over the course of 2013,

and for the considered input they have given to shaping a new programme of work that has been formulated for 2014-17.

I hope that you enjoy reading this review and welcome your feedback on its style and contents, and on the output of our work.





## Note from the Government Chemist Working Group (GCWG)

I am very pleased to contribute to the 2013 Government Chemist Review as Chair of the NMO Government Chemist Working Group, GCWG.

The governance of the Government Chemist Programme is a key function of the GCWG. Stakeholders made up of regulatory and policy officials, representatives from industry, Public Analysts, Port Health Authority officials and academics meet twice a year to provide independent scrutiny of referee casework, research and advice given by the Government Chemist. The group also reviews the quarterly progress reports. This year we convened a Decision Conference to prioritise the capability building work of the Government Chemist to be funded under the forthcoming 2014-2017 Programme.

The work carried out as reported in this review continues to demonstrate an impressive diversity of measurement techniques,

referee cases, advisory work and research, which is disseminated widely and welcomed by all stakeholders. As the former Chief Executive of a leading food research organisation I appreciate only too well the ever increasing complexity of measurement science and the interpretation of findings in this area. Thus the safeguards offered by the Government Chemist to underpin the probity of official controls continue to add value. This can be seen particularly well in the work carried out following the “horsemeat scandal” early in 2013.

In the context of the diversity and complexity of the scientific and legal issues arising in our deliberations I am especially grateful for the continuing hard work and dedication of my colleagues in the working group. It is also gratifying to note that our input and advice is appreciated and regarded as helpful both by the National Measurement Office and the Government Chemist.

**Professor Paul Berryman**  
BSc, MChemA, PhD, MBA, FRSC, CSci  
Chair, Government Chemist Working Group



# 1 Remit

The Government Chemist was created in 1842, originally to help in the protection of the public from fraud, malpractice and harm. The function of the Government Chemist dates from 1879, and is linked to the Food and Drug Acts of that year. The role continues to this day.

The Government Chemist has always used up-to-date and authoritative measurement procedures coupled with interpretative skills to act as a fair and independent arbiter to resolve disputes, to provide public protection and to contribute to effective and efficient regulatory enforcement in industrial sectors where chemical measurements are important. The need to develop measurement techniques and procedures both within our own laboratories and in collaboration with other expert organisations continues to exist. This will enable the Government Chemist to be able to respond to potential future issues as and when they arise.

The Government Chemist fulfils two functions, funded by the Department of Business, Innovation and Skills (BIS).

## Statutory function

The Government Chemist has a statutory function comprising science-based duties prescribed under seven acts of Parliament. These duties (Box 1 on page 7) cover public protection, safety, health, value for money, and consumer choice. Scientific dispute resolution is the most important part of our work; this is usually known as 'referee analysis'. We resolve disputes between regulators and businesses based upon our independent measurements and expert opinion. This is often done without recourse to legal process, which reduces the burden on public finances. Many of these cases are important and can have a significant impact on either or both parties, and so credibility of the referee rests on first-class science, which is underpinned by the assignment of our home laboratory, LGC, as the UK's designated National Measurement Institute for chemical and bioanalytical measurement.

Legislation covering the food, agriculture and medicinal products sectors, where the safety of the consumer is of prime importance,

contains equivalent provisions for the taking of official samples and subsequent analysis.

There are several routes for referral to the Government Chemist. The main route is The Food Safety (Sampling and Qualifications) Regulations 2013 (and their equivalent in Scotland), which are invoked for many of the dispute resolution activities we undertake. These regulations state that all test samples are divided into three parts by an authorised officer. The enforcement authority and Food Business Operator (FBO) – 'the trader' - each receive one of these samples to perform independent analyses, while the third part of the sample is retained in case there is a dispute requiring the Government Chemist to act as referee.

In some circumstances a FBO may request a referral to the Government Chemist without having their own portion of the sample analysed (a procedure known as 'supplementary expert opinion' - described on our website). For businesses, a successful

appeal to the Government Chemist may avoid the effects of penalties prescribed under criminal law, potentially expensive compliance actions and, most seriously, loss of reputation and goodwill. Lastly, the referral sometimes comes from the court itself, with proceedings suspended pending the outcome.

When the Government Chemist's findings confirm those of the enforcement authority, the appropriate action to protect the public can, of course, proceed with increased authority. But, regardless of the outcome, the scientific outputs of the case can be disseminated to all parties and the lessons of these can hopefully be taken on board which should help reduce the possibility of recurrence. Dissemination of referee cases also takes place through scientific publications, seminars, workshops, training events and via our website, [www.governmentchemist.org.uk](http://www.governmentchemist.org.uk).

► **Section 2 of this review looks at the year's completed referee cases.**

Emerging and complex areas are those where the need for referee analysis is often the greatest. The R&D undertaken by the Government Chemist needs to be targeted in these areas, alongside developing areas of analytical measurement which may support these. This enables us to respond better to demands for our services, so we can use the appropriate analytical measurement techniques and complete cases in shorter timescales. The capability developed in this regard is disseminated to stakeholders which should lead to the prevention as well as the resolution of disputes.

► **See Section 3 for an overview of R&D activities**

## Advisory function

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. 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.

Following the privatisation of the Laboratory in 1996, an agreement was signed between the Secretary of State for Trade and Industry and LGC which underpinned the continuity of the broader public functions by appointing the Government Chemist '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'. This agreement is still in place today and continues to highlight the importance of chemical and biochemical measurements in underpinning the UK economy. As new technologies become increasingly adopted, advice is needed to ensure that this is done in an appropriate manner.

The advisory function is delivered principally by responding to government calls for advice or published consultations, where there is a significant analytical science dimension. These responses provide relevant information specifically to the department, agency or body issuing the consultation, and also to a broad range of stakeholders who have an interest in regulatory compliance and the associated measurement implications of this. Consultation Responses are published through the Government Chemist website. The advisory function also looks at emerging issues involving new regulation and related analytical measurements and addresses these by means of small targeted projects and publications, or by publication through the Government Chemist blog<sup>1</sup>.

► **See Section 3 for more about the wider advisory function.**

## Governance

The Department for Business, Innovation and Skills (BIS) funds a programme to enable delivery of statutory casework, scientific advice and any work and research necessary for the ongoing effectiveness of the Government Chemist's functions. Within BIS, responsibility for both the Government Chemist and the wider UK National Measurement System rests with the National Measurement Office (NMO).

Arrangements are in place to ensure that the Government Chemist programme is delivered competently, and that scientific standards, impartiality, transparency and integrity are maintained. LGC has in place rigorous structures and procedures to ensure no conflicts of interest arise between work carried out under the statutory function and its commercial food analysis activities. The Government Chemist Working Group, GCWG, also plays a key role in the governance of the Government Chemist Programme.

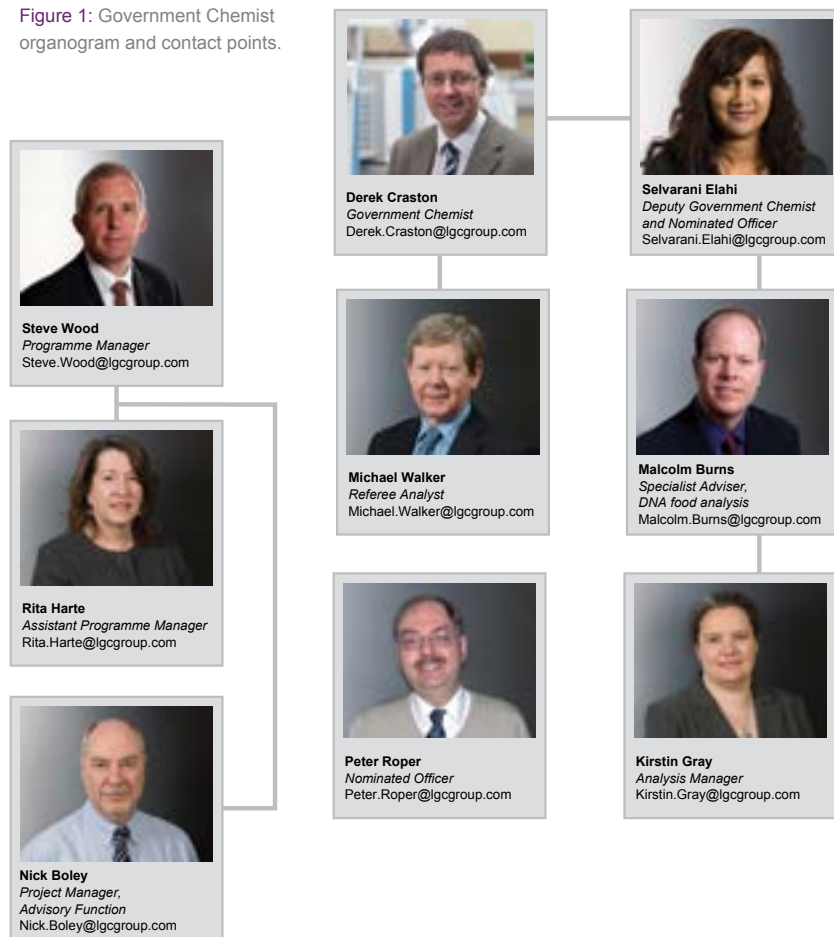
The group provides the necessary independent scrutiny of the programme and offers advice to the NMO regarding future priorities. The GCWG meets twice a year to oversee and discuss the delivery, planning and quality of the programme, and also has oversight of the scientific standards of the programme. The GCWG is tasked by the NMO to advise on:

- The effectiveness and impact of the Programme in providing an independent, expert service to resolve disputes between food control authorities and food traders on analytical results and their interpretation;
- The medium to long term Government Chemist capability building work aimed at preventing disputes arising and enabling better response to referee cases;
- The progress of the current projects in meeting technical milestones and targets; and
- The formulation and prioritisation of new projects to maintain and develop the capabilities needed to discharge the GC functions (i.e. Capability Building, Knowledge Transfer, Regulatory Foresight and Statutory Analysis).

The GCWG comprises representatives of regulatory and enforcement bodies, industry, trade associations and academia, with a broad range of backgrounds, skills and interests.

<sup>1</sup> <http://governmentchemist.wordpress.com/>

Figure 1: Government Chemist organogram and contact points.



<sup>2</sup> All work is overseen by Michael Walker, a nominated officer holding the statutory MChemA qualification

## The current Government Chemist programme

The current Government Chemist programme, covering 2011-2014, commenced in April 2011. The programme reflects the prioritisation exercise carried out by the GCWG, and is similar in structure and themes to the 2008-2011 programme:

- **Intelligence gathering:** horizon-scanning projects on the scientific implications of policy development, emerging legislation, changes to existing legislation and enforcement trends
- **Capability building:** innovative and relevant R&D which aims to reflect potential needs for future casework under the Government Chemist's statutory role
- **Statutory activities:** work carried out in relation to individual cases that are referred to the Government Chemist under his statutory function as defined in Acts of Parliament
- **Knowledge transfer:** improved dissemination of regulatory and analytical developments to a wide range of stakeholders, to stimulate improvement of standards of measurements, the understanding of the regulatory environment and to help industry to innovate concerning new products and processes.

Work is well advanced to develop and plan the next Government Chemist programme, due to commence in April 2014 and run through to 2017. The Government Chemist Working Group has held a Decision Conference to decide which Capability Building Projects should be taken forward, and agreed the proposed projects covering Regulatory Foresight, the Statutory Function, Knowledge Transfer and Programme Management. The planning for the new programme has also looked at effective ways to improve communications through the modern mechanism of social media.

## People

LGC staff who directly support the Government Chemist function have clearly and independently defined roles (Figure 1). Within this framework, there are particular requirements for the management of statutory casework:

- Nominated officers, one of whom holds the requisite statutory qualification<sup>2</sup>, have overall responsibility for case supervision. They prepare and sign Government Chemist certificates of analysis;
- Only the Government Chemist or Deputy, once satisfied that the case has been properly completed, may countersign.

The members of staff carrying out work under the Government Chemist's statutory function must continually demonstrate their competence through participation in an extensive variety of appropriate proficiency testing schemes and collaborative studies. The diverse nature of LGC's scientific activities therefore leads to a wide range of skills and specialisms being available in-house. Many of the staff involved in delivering the programme have also carried out research and development work, which often involves programmes with international collaboration, which gives them the capability to contribute positively and efficiently to their work.



## Collaboration

The work we carry out within the Government Chemist programme is by necessity very broad. We are not always fully equipped to undertake all the tasks within the programme which we consider to be appropriate. Consequently there is sometimes a need to collaborate with stakeholders on specific areas of work, and we are always happy to do so. We also constantly seek new stakeholders to become involved in our work so that we are better able to respond to the future challenges of potential casework. Our work with the University of Kingston on the development of an online network analysis tool for the real-time evaluation of global food notifications is a good example of this approach. Thus our capability building research utilises a broad range of expertise which will benefit public health, safety and well-being, as well as the wider scientific community, including those UK manufacturing industries which depend on reliable and accurate analytical measurement, and how they interact with regulation.

If you would like to get involved with any aspect of our work, or for more information on our work, please contact us at [Government.Chemist@lgcgroup.com](mailto:Government.Chemist@lgcgroup.com) or go to the website ([www.governmentchemist.org.uk](http://www.governmentchemist.org.uk)).



### Box 1: The Government Chemist in legislation

#### The duties of the Government Chemist as referee analyst are defined in or under:

Food Safety Act 1990  
Food Safety (Sampling and Qualifications) Regulations 2013  
Food Safety (Sampling and Qualifications) (Scotland) Regulations 2013  
Food (Northern Ireland) Order 1989  
Food Safety (Northern Ireland) Order 1991  
Food Safety (Sampling and Qualifications) Regulations (Northern Ireland) 2013  
Poultry Meat (Water Content) Regulations 1984  
Natural Mineral Water, Spring Water and Bottled Drinking Water Regulations 2007  
Materials and Articles in Contact with Food (England) Regulations 2012  
Materials and Articles in Contact with Food (Scotland) Regulations 2012  
Materials and Articles in Contact with Food (Wales) Regulations 2012  
Materials and Articles in Contact with Food (Northern Ireland) Regulations 2012

Agriculture Act 1970  
Feed (Hygiene and Enforcement) Regulations 2005  
Genetically Modified Animal Feed Regulations 2004

Human Medicines Regulations 2012  
Farm and Garden Chemicals Act 1967

#### The Government Chemist is named and has other scientific responsibilities under:

Merchant Shipping Act 1995  
Hydrocarbon Oil Duties Act 1979  
Poisons Act 1972

#### The status and territorial extent of the Government Chemist are understood with reference to:

Freedom of Information Act 2000  
Scotland Act 1998 (Cross-Border Public Authorities) (Specification) Order 1999  
Administrative Provisions Act (Northern Ireland) 1928  
Government Chemist Regulations (Northern Ireland) 1928



## 2 Science underpinning sound dispute resolution

Referee casework arises most frequently under the Food Safety Act 1990 or the Agriculture Act 1970.

Formal samples taken under statutory enforcement provisions are divided into parts for analysis on behalf of the authorities, the food and feed business operator (FBO) and, when required, the referee. During 2013, 18 cases were referred to the Government Chemist, all in connection with the Food Safety Act. Further information about some of these cases is presented later in this section.

### The Referee Function

A wide variety of problems were referred to us in 2013. Scientifically they ranged over analytical, physical, organic and inorganic chemistry as well as molecular biology. The matrices included meat products, fish, nuts, beans, noodles and kitchen utensils in which we resolved disputes on meat species, carbon monoxide, polycyclic aromatic hydrocarbons, aflatoxins, pesticides, aluminium, formaldehyde and primary aromatic amines. In all 18 cases were dealt with.

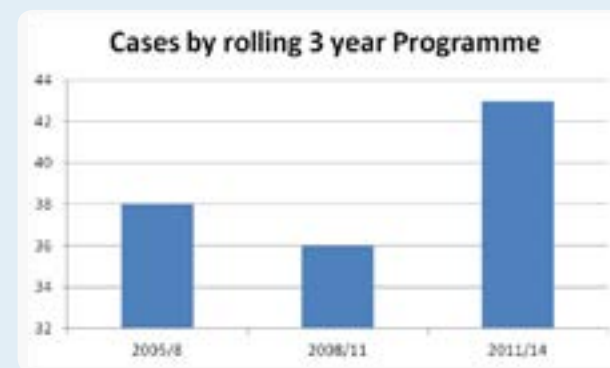
**Table 1** Overview of Referee Cases in 2013

Origin			Basis		
Inland Authority	8	44 %	Dispute	13	72 %
Port Health Authority	10	56 %	SEO*	5	28 %

SEO – Supplementary Expert Opinion, pursuant to Article 11(5) of Regulation 882/2004 *on official controls*

Referee casework is a demand led service which has been at the core of the Government Chemist's function since 1875 and demand remains high – see Figure 2.

In guaranteeing fair scientific treatment for all by authoritative adjudication on disputes we underpin public confidence in food and feed official control systems. We maintain the even-handed



**Figure 2:** Referee Cases (to date) in each of the three recent 3 year GC Programmes

credibility of this referee role by stringent governance of the function and painstaking analytical rigour. Our aim is to safeguard consumers, regulators, the agrifood sector and the courts from unwitting errors in measurement science.

Referee casework arises most frequently under the Food Safety Act 1990 and the Agriculture Act 1970. Formal samples taken under statutory enforcement provisions are divided into parts for analysis on behalf of the authorities, the FBO and, when required, the referee.

Analytical results must be interpreted in increasingly complex scientific legal and policy contexts, and in an increasingly global supply chain. When a referral is received we begin with a case meeting to examine the problems associated with the case and instigate a literature review in the area if it is new to us. Our default analytical strategy is multi-replicate analyses on multiple days. The extent of replication together with analysis of certified reference materials, where available, and of blanks and spiked blanks provide an exceptionally high level of analytical confidence. All significant analytical steps are witnessed by a second scientist and the results are evaluated against prescribed quality control criteria. The entire dataset is independently evaluated by professional statisticians for bias and outlying results and to yield a case-specific measurement uncertainty. A certificate is drafted and reviewed by a qualified person and finally the case file is brought to the Government Chemist for peer review. If all steps are satisfactory the Government Chemist will allow the findings to be released usually in the form of a certificate. Along with the high-end equipment deployed, these measures are aimed to give the food business owner, the courts and regulators the necessary assurance that the appellate function is discharged to the highest possible professional standards.

## Carbon Monoxide – food fraud or naturally occurring?

Carbon monoxide, CO, is an odourless, colourless toxic gas formed, for example, as a stable product of incomplete hydrocarbon combustion. Carbon monoxide poisoning is a well known phenomenon; fatalities continue to occur with tragic regularity. Less well known is the fraudulent use of CO to deceive consumers about the freshness of some fish.

The freshness of fish such as tuna can be judged by its bright red colour which is caused primarily by the red pigment (oxy-myoglobin) formed by reaction of oxygen with myoglobin, a heme protein that with haemoglobin is essential for the storage and transport of

oxygen. However, oxidation of the ferrous iron in oxy-myoglobin to the brown ferric met-myoglobin gradually changes the fish colour to various shades of brown. This can be prevented by CO which reacts with the oxy-myoglobin to form a very stable cherry red carboxy-myoglobin complex, figure 2. But the carboxy-myoglobin colour persists beyond the microbiological shelf life of the fish and also potentially masks the proliferation of toxic histamine<sup>3</sup>. CO is therefore not permitted to be added to food and is not included in the EU list of permitted food additives<sup>4</sup>.



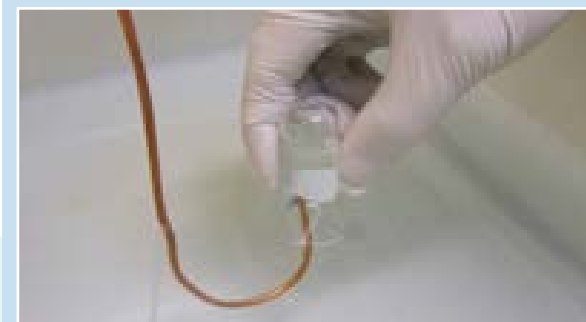
**Figure 2** Tuna exposed to CO in the laboratory and showing the cherry red carboxy-myoglobin pigment

In late 2012 and early 2013 three consignments of tuna were referred to the Government Chemist by a trader who disputed the Public Analyst's findings of CO in the fish. We carried out our investigation by gas chromatography mass spectrometry, GC-MS, using a molecular sieve column, monitoring the CO ion at  $m/z$  28<sup>5</sup> and used the ideal gas law to calibrate our equipment Box 2.

In each case we confirmed the Public Analyst's findings that the fish contained CO. However literature investigations also revealed that there is endogenous production of CO in the blood during heme catabolism by oxygenases<sup>6</sup>. In fact Anderson and Wu<sup>5</sup> reported data on CO concentrations in fish not knowingly treated with CO (see

Figure 3). Dialogue with the Food Standards Agency who consulted the European Commission, confirmed that CO treated products have consistently shown a CO content above 0.2 mg kg<sup>-1</sup> <sup>7</sup>. The mean concentrations of CO found in the samples we examined fell below 0.2 mg kg<sup>-1</sup> and within the range of naturally occurring concentrations. Hence we concluded that carbon monoxide was not used as a non-permitted additive in the samples but was naturally occurring. Our findings and method details were made available to both parties. It is important that consumers are protected from fraudulent use of CO to disguise deterioration of fish and, as always, confidence in regulation hinges on correct analysis and informed interpretation.

### Box 2 Determination of Carbon Monoxide in fish



Carbon monoxide collected over water.

Standard Preparation: 1, 5, 10, 50, 100, 150  $\mu$ L  $\rightarrow$  20 mL,  
100  $\mu$ L manually  $\rightarrow$  GC-MS

Calibration by the equation of state for a perfect gas

$$PV = nRT$$

Where  $P$  = Pressure,  $V$  = Volume,  $n$  = amount of carbon monoxide (moles of CO)

$R$  = the ideal Gas Constant and  $T$  = Temperature,  $^{\circ}$ K

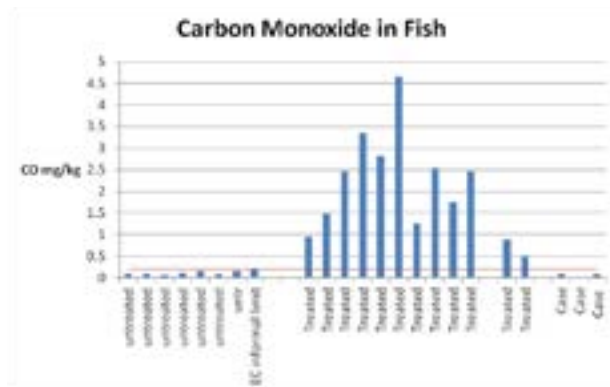
<sup>3</sup> Giulietta Smulevich, Enrica Droghetti, Claudia Focardi, Massimo Coletta, Chiara Ciaccio, Mila Nocentini, 2007, A rapid spectroscopic method to detect the fraudulent treatment of tuna fish with carbon monoxide, *Food Chemistry* 101, 1071–1077

<sup>4</sup> Article 4(1) of Regulation No 1333/2008

<sup>5</sup> CR Anderson and Wen-Hsin Wu, 2005, Analysis of Carbon Monoxide in Commercially Treated Tuna (*Thunnus spp.*) and Mahi-Mahi (*Coryphaena hippurus*) by Gas Chromatography/Mass Spectrometry, *J. Agric. Food Chem.*, 53: 7019–7023

<sup>6</sup> Lingyun Wu And Rui Wang, 2005, Carbon Monoxide: Endogenous Production, Physiological Functions, and Pharmacological Applications, *Pharmacol Rev* 57:585–630, 2005

<sup>7</sup> Personal communication, G Griffiths email to M Walker 20.02.2013



**Figure 3:** Concentrations of CO in fish, literature values and casework results. Horizontal line shows 0.2 mg kg<sup>-1</sup> informal limit differentiating CO untreated from CO treated fish

## Horse meat

On 15 January 2013 the Food Safety Authority of Ireland (FSAI) published a press release on a small survey identifying horse and pig DNA in burger products. This initiated a meat substitution scandal that involved most of Europe and maintained high and lengthy media and political salience. The 1991 edition of 'Pearson's Composition and Analysis of Foods' contains the statement that "Horse meat has long been a common substitute for beef"<sup>8</sup>. Although there is a vast literature on species detection methods, well documented episodes of species substitution are sparse, hence we recorded the 2013 episode extensively and in open access format elsewhere<sup>9</sup>. The Government Chemist was asked to investigate 7 formal samples for which traders had lodged objections against the Public Analysts' findings. Table 2 summarises these cases.

In 6 out of 7 cases (86 %) we confirmed the various Public Analysts' findings. We deployed both polymerase chain reaction DNA, PCR DNA, and enzyme linked immunosorbent assay, ELISA, methods.

Sample	Alleged Foreign Species	GC Findings
Beefburger	Horse	Horse tissue confirmed present
Beefburger	Pork	Pork tissue confirmed present
Beef sausages	Pork	Pork tissue confirmed present
Beefburger	Horse	Horse tissue confirmed present
Beefburger	Horse	Horse tissue confirmed present
Beef cheek meat	Sheep	Sheep tissue confirmed not detected
Cooked Strips of Beef Meat	Horse	Horse tissue confirmed present

DNA was extracted from homogenised 1 gram aliquots by incubation and lysis of cellular components in a sodium dodecyl sulphate (SDS) buffer with Proteinase K, binding of isolated DNA to positively charged silica beads, followed by multiple washing steps and an ethanol precipitation to clean, elute and concentrate the extracted DNA.

Equine (*Equus* genus) nuclear DNA and pork (*Sus* genus) nuclear DNA were tested for by real time PCR by the method of Köppel *et al.* The assays applied have been validated for specificity against a range of 14 common meat species and 35 herbs, spices, nuts and cereals with cross reactivity to horse (*Equus caballus*) only observed for mule (*mulus*) and donkey (*Equus asinus*) and no cross reactivity observed for pork. Mitochondrial DNA was also analysed by commercially available Real Time PCR kits designed to detect horse (*Equus caballus*) and pig (*Sus scrofa*). Two real-time PCR instruments were deployed, the Applied Biosystems™ 7900HT Fast Real-Time PCR System (ABI 7900) and the BIO-RAD CFX™ Real-Time PCR System (BioRad).

and examined by ELISA for heat resistant horse species-specific muscle related glycoproteins, and for heat resistant pork species-specific proteins.

Interpretation of all DNA and ELISA results as detected or not detected was relative to the validated LOD of the respective assays. This was based on best measurement practice and kit manufacturer's instructions, where applicable. Interpretation of results obtained by all of the above approaches was aided by concurrent analysis of gravimetric reference mixtures, prepared under controlled conditions e.g. at 1 % w/w, 0.5 % w/w and 0.1 % w/w of raw horse in raw beef or raw pork in raw beef, figures 4, 5, 6 and 7. From these it was possible to *infer* what the response from PCR amplification of DNA might reflect in terms of the likely equivalent w/w material on the assumption that tissue types are matched exactly between test sample and gravimetric mixture. This approach does not have general applicability and a quantitative DNA/DNA approach is the subject of current validation work.

<sup>8</sup> R S Kirk & R Sawyer, Pearson's Composition and Analysis of Foods, 1991, 9th Ed. Longman Scientific & Technical, Essex, UK. P. 492

<sup>9</sup> Michael J. Walker, Malcolm Burns and D Thorburn Burns, 2013, Horse meat in beef products, species substitution, 2013, J. Assoc Public Analysts (online), 41, 67 – 106 [http://www.apajournal.org.uk/html/japa\\_vol\\_41\\_pg\\_67-106.html](http://www.apajournal.org.uk/html/japa_vol_41_pg_67-106.html)



Reference materials of authenticated meat species and gravimetric mixtures of same are available from LGC Standards. *Post hoc* DNA sequencing confirmed the species identity in each referee case<sup>10</sup>. The GC certificate reports contained only tentative assignment of the levels of contamination.

There are difficulties in quantifying, by ELISA and DNA approaches, any one meat species in admixture with other species and ingredients. These were, for DNA, summarised in 2010 by Primrose *et al.*<sup>24</sup>. It cannot be assumed that the amount of DNA present is a true reflection of the amount of meat present because the DNA may have been degraded during processing which may also, along with other ingredients affect the amount of DNA that can be extracted. Clearly these considerations impact upon processed foods e.g. ready meals that are multi-ingredient and cooked for sale much more than e.g. 'all beef' beefburgers sold raw where ground skeletal muscle (of whatever species) is the main ingredient. Moreover in real-time PCR, the copy number of the marker gene (for the adulterant) is measured and compared to the copy number of a 'normalising' gene. This ratio can then be compared, with caution, to results from standard mixtures to infer the amount (e.g. percentage) of the adulterant. PCR suffers from inhibition and amplification efficiency issues due to matrix effects which can influence these calculations and although the variation is less problematic for foods with a limited number of ingredients, the results for composite ingredient foods have been found to be very variable<sup>11</sup>.



Figure 4: Horse meat being prepared for testing and for standard reference material production



Figure 5: Horse meat being prepared for testing and for standard reference material production



Figure 6: Single species Reference Material Horse meat



Figure 7: Horse meat gravimetric mixtures

<sup>10</sup>Claire Bushell and Malcolm Burns, 2013, A PCR and sequencing approach for the detection of horse DNA in food products, *paper in preparation to provide practical advice and guidance to the laboratory analyst on how to use PCR and DNA sequencing for the detection of horse DNA in food products, illustrated with a realistic worked example.*

<sup>11</sup>Hird, H., Saunders, G., Popping, B., Garrett, S., & Wiseman, G., 2005, Final optimisation and evaluation of DNA based methods for the authentication and quantification of meat species, Project Q01084, Available in FSA Foodbase at [http://www.foodbase.org.uk/admin/tools/reportdocuments/267-1-488\\_QO1084\\_species\\_id\\_final\\_report.pdf](http://www.foodbase.org.uk/admin/tools/reportdocuments/267-1-488_QO1084_species_id_final_report.pdf) accessed 08.07.2013

The main problem, for DNA methods lies in the tension between detection and quantification. Some of the genes that are targeted in authenticity tests are located on mitochondrial DNA. Mitochondria are organelles that produce energy through oxidative phosphorylation, are involved in many cellular processes<sup>12</sup> and are present as multiple copies in each cell. Copy numbers in the range 1000 – 8000 per mammalian cell, including multiple copies within each mitochondrion are typical<sup>13, 14</sup>. Although this makes mitochondrial detection much more sensitive compared with that of nuclear genomic DNA, particularly in highly processed samples, it makes exact quantification almost impossible because many factors can influence organelle copy number and as a result the copy number varies within the tissues of a single animal and from one animal to another. Ballin *et al.* reviewing species determination in food and feed by ELISA and DNA approaches confirm the above, note that genome size is also an influence (e.g. 3-fold size difference between the chicken and cow genome) as are tissue-variable fat and water contents<sup>15</sup>. These authors recommend that quantitative species determination should be by real time PCR of genomic DNA expressed as genome/genome equivalents rather than on a weight/weight (w/w) basis.

Thus the majority of DNA approaches for meat speciation are qualitative in nature: that is, the species specific DNA fragment will either be reported as detected or not detected, with an associated limit of detection (LOD). There is currently no officially recognised, standardised or approved approach for quantifying the levels of meat species adulteration, and although this state of affairs is by no means uncommon in food standards work, opinion is also divided between expressing results in terms of w/w tissue measurements or DNA/DNA copy numbers. Whilst

the former may help to promote understandable results in line with public interest and conforms to the European Union' view of defining threshold levels for prohibited/adulterant species, the latter is scientifically more achievable and traceable. As described above there is no direct conversion between DNA/DNA and w/w tissue measurements, and such a comparison is significantly affected by many factors including species, genome size, tissue type, matrix background, other ingredients, processing, level of degradation, and PCR efficiency.

Despite the above limitations, DNA approaches for meat speciation appear to be preferred because of potential advantages over protein detection methods, including specificity, sensitivity, the presence of DNA in virtually all tissue types, choice of targets and potential for development of a quantitative estimate without the risk of saturation (of antibody). By including standard w/w mixtures of specified species in the analytical procedure it is possible to infer an approximate w/w composition in the target sample however this inference should currently only be used for illustrative purposes as, in addition to the caveats rehearsed above, it relates solely to the tissue used to prepare the w/w reference mixtures and their use cannot necessarily be extrapolated to the analysis of other mixtures.

The Food Standards Agency reported on the investigations by the Government Chemist<sup>16</sup> and a supporting statement was placed on the GC website<sup>17</sup>.

The variation in nuclear and mitochondrial DNA prevalence by species and tissue type is analogous to that for nitrogen content. A method based on the determination of the nitrogen content of a sample and its comparison, corrected for non-flesh nitrogen,

with the species specific nitrogen concentration, the 'Nitrogen Factor' has been used to quantify meat and fish ingredients in food since the inception of the technique by the Government Chemist in 1919<sup>18</sup>. Validated data for nitrogen factors of meat, poultry and fish have been produced by the Analytical Methods Committee of the Royal Society of Chemistry and the Association of Public Analysts, with input from LGC and trade laboratories for many years<sup>19</sup>. An option would be to investigate DNA variability in a similar manner.



<sup>12</sup> Lars Ernster and Gottfried Schatz, 1981, Mitochondria: A Historical Review, J Cell Biology, 91, 227s -255s

<sup>13</sup> R Saunders Williams, 1986, Mitochondrial Gene Expression in Mammalian Striated Muscle, J Biol Chem, 261, 12390 - 12394

<sup>14</sup> Erika Fernández-Vizarra, José A. Enríquez, Acisclo Pérez-Martos, Julio Montoya, Patricio Fernández-Silva, 2011, Tissue-specific differences in mitochondrial activity and biogenesis, Mitochondrion 11, 207–213

<sup>15</sup> Nicolai Z. Ballin, Finn K. Vogensen, Anders H. Karlsson, 2009, Species determination – Can we detect and quantify meat adulteration? Meat Science, 83, 165–174

<sup>16</sup> Food Standards Agency, Update on Agency's beef product survey, 9 April 2013, available at <http://www.food.gov.uk/news-updates/news/2013/apr/survey-update> last accessed 02.02.2014

<sup>17</sup> Government Chemist, Horsemeat cases: supporting statement on behalf of the Government Chemist, 16 April 2013, available at <http://www.governmentchemist.org.uk/News.aspx?m=2&amid=1667> last accessed 02.02.2014

<sup>18</sup> G. Stubbs and A. More, (1919), "The estimation of the approximate quantity of meat in sausages and meat pastes", Analyst, 44, 125-127

<sup>19</sup> D. Thorburn Burns, M Walker, S. Elahi and P. Colwell, 2011, Nitrogen factors as a proxy for the quantitative estimation of high value flesh foods in compound products, a review and recommendations for future work, Anal. Methods, 3, 1929

## Food Contact Materials

Food Contact Materials (FCM) containers, packaging, cutlery, dishes, and any materials and articles that come into contact with food or water used in cooking or preparation of food, can transfer components into food. Materials such as plastics, paper and board, metals and ceramics are commonly used for the manufacturing of food packaging, but also kitchenware and cutlery and food processing machinery. To protect public safety and consumer confidence, FCM must not transfer their components into the foods in unacceptable quantities with regard to safety or organoleptic properties. Legal limits are set on the transfer or 'migration' of specific compounds and there are also generic limits for non-specific, or 'overall' migration.

During 2013 two cases were referred on FCM, one a melamine: formaldehyde polymer plate and bowl set and the other a set of kitchen utensils (e.g. potato mashers, slotted turners and spoons and ladles). The compounds and measurements in dispute were formaldehyde and aniline, a primary aromatic amine, respectively.

The thermosetting plastic melamine resin is used to manufacture a diversity of inexpensive food contact articles intended for repeated use, such as picnic sets, children's cups and plates often printed with popular motifs, and in kitchenware such as bowls and ladles. Melamine is a polycondensation product of the monomers formaldehyde and melamine and residues of both may remain in the finished product. Thus both compounds are on the EU monomer positive list with specific migration limits<sup>20</sup>.

Formaldehyde, H<sub>2</sub>C=O, average mass: 30.026 Da, is an interesting example of a compound that occurs in food both from natural and man-made sources. Its toxicology is complex; it is known to be capable of sensitising some people to allergic contact dermatitis

and there is evidence that it is a carcinogen.<sup>21,22</sup>

Primary aromatic amines, PAAs, where one hydrogen from ammonia is replaced by an aromatic group (e.g. aniline, figure 8) are very useful chemicals. PAAs are not intended to be present in the final product, but residual amounts may be found from incomplete reaction, from impurities, by-products, or degradation products. Many PAAs are toxic and/or suspected human carcinogens. The combination of a potentially high exposure and high toxicity means the use of these chemicals is extensively regulated<sup>23</sup>. In recent years, via the RASFF, many notifications have been issued for high migration of PAAs from polyamide (nylon) kitchen utensils imported from the Far East.

Regulation (EU) No 284/2011 lays down conditions and procedures for the import of polyamide and melamine plastic kitchenware originating in or consigned from the People's Republic of China and Hong Kong Special Administrative Region, China. The vires of the Government Chemist to act in relation to FCM derives from successive national measures<sup>24</sup> on Materials and Articles in Contact with Food implementing and enforcing a group of European Directives and Regulations designed to protect consumers' health and remove technical barriers to trade<sup>25</sup>.

The analysis in both cases was informed by guidance issued by the European Reference Laboratory for Food Contact Materials.

Formaldehyde migration was determined by exposure of the plastic material at 70° C for two hours to 3 % v/v aqueous acetic acid food simulant to mimic the worst case scenario in actual use. An iodometric titration against the primary standard potassium iodate was used to characterise a stock calibrant solution of formaldehyde. The final analytical step applied two well known reactions, one with chromotropic acid and the other with pentane-2,4-dione (acetylacetone) stoichiometrically to produce coloured

reaction products for the spectrometric determination of migrated formaldehyde<sup>26</sup>. Since the test items were intended for repeated use each item was exposed to fresh food simulant in three consecutive tests with formaldehyde concentrations reported from the third (final) exposure of each test specimen. In each instance three replicates were analysed alongside exposed food simulant spiked with formaldehyde and blank (unexposed) simulant. As is usual a case specific measurement uncertainty was derived and applied in appraisal of the results.



Figure 8: Nylon kitchen materials as received

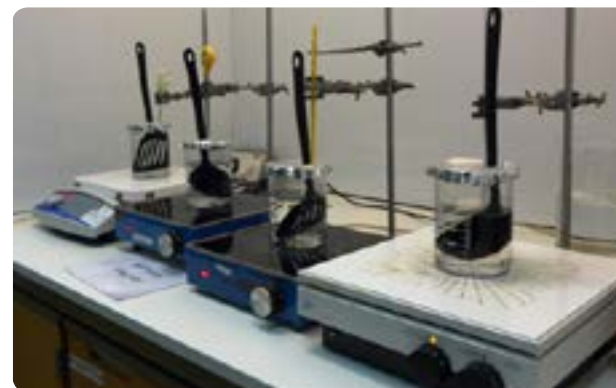


Figure 9: Nylon kitchen materials undergoing migration testing

<sup>20</sup> K. H. Lund, J. H. Petersen, 2006, Migration of formaldehyde and melamine monomers from kitchen- and tableware made of melamine plastic, *Food Additives and Contaminants*, 23, 948-955

<sup>21</sup> Lois Lehman-McKeeman, 2010, Paracelsus and Formaldehyde 2010: The Dose to the Target Organ Makes the Poison, *Toxicol. Sci.* 116, 361-363

<sup>22</sup> Hermann M. Bolt, Peter Morfeld, 2012, New results on formaldehyde: the 2nd International Formaldehyde Science Conference (Madrid, 19–20 April 2012), *Arch Toxicol.* 87, 217–222

<sup>23</sup> Sarah Kelly Mortensen, Xenia Thorsager Trier, Annie Foverskov, Jens Højslev Petersen, 2005, Specific determination of 20 primary aromatic amines in aqueous food simulants by liquid chromatography–electrospray ionization-tandem mass spectrometry, *J Chromatog A*, 1091, 40 – 50

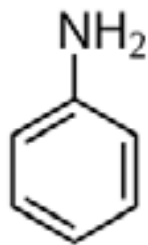
<sup>24</sup> The Materials and Articles in Contact with Food (England) Regulations 2012, No. 2619, the Materials and Articles in Contact with Food (Scotland) Regulations 2012, No. 318, the Materials and Articles in Contact with Food (Wales) Regulations 2012, No. 2705 (W. 291) and the Materials and Articles in Contact with Food Regulations (Northern Ireland) 2012, No. 384

<sup>25</sup> European Commission, Food Contact Materials, [http://ec.europa.eu/food/food/chemicalsafety/foodcontact/index\\_en.htm](http://ec.europa.eu/food/food/chemicalsafety/foodcontact/index_en.htm) accessed 29.01.13

<sup>26</sup> According to CEN/TS 13130-23, Materials and articles in contact with foodstuffs – Plastics substances subject to limitation – Part 23: Determination of formaldehyde and hexamethylenetetramine in food simulants.



Of the PAAs, only the migration of aniline was in dispute in the related case, hence six of each of the kitchen utensils (spoons, slotted turners, ladles and slotted spoons) were tested, one of each type of utensil on each of six separate days. Migration tests were conducted using food simulant B (3% v/v aqueous acetic acid), which was considered most appropriate in this situation. Since the test items are intended for repeated use each item was exposed to fresh food simulant in three consecutive tests. Following temperature profiling experiments, covered test specimens were exposed to food simulant so that the simulant was maintained at close to 100°C for two hours. The exposed simulant was analysed by gradient elution liquid chromatography-tandem mass spectrometry (LC-MS/MS) monitoring the transitions m/z 94 to 77 and 94 to 51 which are characteristic of aniline. Again, exposed food simulant spiked with aniline and blank (unexposed) simulant were analysed and the resulting dataset subject to statistical analysis.



**Figure 10:** Aniline, a primary aromatic amine<sup>27</sup>

Article 11 and Annex I of Commission Regulation (EU) 10/2011 limit the transfer of formaldehyde from FCM to a maximum of 15 milligrams of formaldehyde per kilogram of food, mg kg<sup>-1</sup> and also prohibit the transfer of PAAs<sup>28</sup> such as aniline in a detectable quantity; a detection limit of 0.01 milligrams of aniline per kilogram of food, mg kg<sup>-1</sup> is defined in Annex II.

In the formaldehyde case our results demonstrated that, of the six bowls analysed, one exhibited transfer of formaldehyde into the food simulant in excess of the permitted maximum of 15 mg kg<sup>-1</sup> whereas five did not. On statistical analysis the amount of migrated formaldehyde was predicted to range from 10.7 to 15.5 mg kg<sup>-1</sup> at a 95 % confidence interval. We thus

concluded that while only one of the tested items did not satisfy the requirements of Regulation 10/2011 overall the sample failed to demonstrate compliance with the Regulation and Regulation 2023/2006 of 22 December 2006 on good manufacturing practice for FCM by way of lack of control to ensure conformity with the maximum of 15 mg kg<sup>-1</sup>.<sup>29</sup>

In the kitchen utensils aniline case all items of one particular sample number tested were well below the regulatory detection limit. The results presented interesting statistics such as non-uniform variance and included zeros, thus classical analysis of variance was unsuitable. More detailed statistical analysis of the data was performed and an estimate of a migrated aniline concentration of 0.084 µg kg<sup>-1</sup> was obtained with a corresponding maximum value at the 95 % confidence level of 0.4 µg kg<sup>-1</sup>. The legislative limit of detection is 10 µg kg<sup>-1</sup> which can be taken as the *de facto* operational limit. Why then did the Public Analyst find that the utensils exhibited a non-compliance for aniline migration whereas the trader's lab did not, particularly in relation to the statistical information obtained and the legislative limit of detection?

LGC statisticians were asked to look at the problem of sampling these items. On the basis of the number of utensils in the formal sample that were tested by the trader, the Public Analyst and the Government Chemist we estimated that 1 utensil in 48 failed for aniline migration where 'failure' means a utensil with an aniline level above the 'limit' of 10 µg kg<sup>-1</sup>. On the assumptions of valid measurement and above-limit utensils randomly selected from a population with random aniline distribution<sup>30</sup> a Poisson distribution shows that to achieve a 95 % likelihood of detecting a non compliance 144 utensils would need to be tested. In the light of which, the disparity between the results obtained by each laboratory is understandable. As an alternative to the expense of statistically rigorous sampling and testing the EU-RL has

recommended<sup>31</sup> sampling and testing 15 articles (or 15 sets of articles), 5 for enforcement purposes, 5 for trade purposes and, where national rules apply as they do in the UK, 5 for referee purposes. In these circumstances resolution of any, in effect statistically driven, dispute between enforcement and trade laboratories by reference to the Government Chemist appears to be a better use of public funds than the sampling and testing of 144 items.

## Pesticides residues

All foodstuffs intended for human or animal consumption in the EU must conform to maximum residue levels (MRLs) for pesticides in order to protect animal and human health<sup>32</sup>. MRLs are a complex issue but in general are recommended by the European Food Safety Authority (EFSA) based on a risk assessment and adopted in law by the Commission. Where a MRL has not been specifically set a 'default' MRL of 0.01 mg kg<sup>-1</sup> is applied. Referee casework in pesticides residues is unusual and for the first time, in 2013, we were asked to look at a dispute between the Public Analyst and a trader in relation to the pesticide dichlorvos in green beans. Dichlorvos, O-(2,2-dichlorovinyl)-O,O-dimethylphosphate (DDVP) is a volatile organophosphorus insecticide. In line with recognised official guidance<sup>33</sup> three replicates of the referee sample were analysed on each of three days by acetonitrile extraction followed by gas chromatography mass spectrometry (GC-MS/MS). Quantification was performed against a standard curve prepared from a blank bean matrix fortified with dichlorvos at appropriate concentrations. We confirmed the Public Analysts findings that dichlorvos was present in the sample at a mean concentration above the MRL of 0.01 mg kg<sup>-1</sup> however when a case specific measurement uncertainty was applied the resulting lower bound datum was just below the MRL and so a non-compliance could not be certified, 'beyond reasonable doubt'.

<sup>27</sup> Source: <http://0.tqn.com/d/chemistry/1/0/o/A/1/Aniline.jpg>

<sup>28</sup> Other than compounds appearing in Table 1 of Annex I of the Regulation, which compounds are not relevant to this matter.

<sup>29</sup> Commission Regulation (EC) No 2023/2006 of 22 December 2006 on good manufacturing practice, GMP, for materials and articles intended to come into contact with food requires business operators to establish GMP listed in Annex I to Regulation (EC) No 1935/2004 to ensure that materials and articles are consistently produced and controlled to ensure conformity with the rules applicable to them. The requirements are to be applied proportionately to avoid undue burdens for small businesses and allow the trader to produce appropriate paper or electronic documentary records to the competent authorities at their request.

<sup>30</sup> A batch or process problem would be a different matter

<sup>31</sup> Technical guidelines on testing the migration of primary aromatic amines from polyamide kitchenware and of formaldehyde from melamine kitchenware, 1st edition 2011, EUR 24815 EN 2011, Ed. C Simoneau

<sup>32</sup> Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC

<sup>33</sup> Method Validation and Quality Control Procedures For Pesticide Residues Analysis in Food And Feed, Document N° SANCO/12495/2011



## Polycyclic Aromatic Hydrocarbons, PAH

PAH constitute a large class of organic compounds that are composed of two or more fused aromatic rings and generally occur in complex mixtures which may consist of hundreds of compounds. PAH are primarily formed by incomplete combustion or pyrolysis of organic matter and during various industrial processes. Human exposure is by various pathways; for non-smokers the major route of exposure is food. Toxicological concern for consumer health at average estimated dietary exposures is gauged to be low but for those consuming significantly higher levels of meats cooked at temperatures above 300 °C there is a potential concern. At least 13 PAH have been assessed as genotoxic and carcinogenic, thus maximum concentrations in food must be safe and as low as reasonably achievable based upon good manufacturing and agricultural/fishery practices. Maximum levels for the sum of four PAH (benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene) have been introduced, whilst maintaining a separate maximum level for benzo(a)pyrene for continuity with historical data. Limits are shown in Table 3.<sup>34,35</sup>

In 2013 we investigated an alleged non-compliance with the above limits in a product known as 'Katsuobushi', a traditional Asian speciality food produced from tuna by boiling, smoking and drying processes. The final product is a very high protein food said to be consumed as stock by mixing slivers of the shaved product in boiling water, and discarding the solids after a few minutes contact. Figure 11 shows typical photograph of an example product.

**Table 3** Current and future maximum limits for PAH in food

Benzo(a)pyrene $\mu\text{g kg}^{-1}$	Sum of Benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene, $\mu\text{g kg}^{-1}$
5.0 until 31.8.2014	30.0 as from 1.9.2012 until 31.8.2014
2.0 as from 1.9.2014	12.0 as from 1.9.2014

**Figure 11:** Katsuoboshi sample



<sup>34</sup> Polycyclic Aromatic Hydrocarbons in Food, Scientific Opinion of the Panel on Contaminants in the Food Chain, adopted on 9 June 2008, *The EFSA Journal* (2008) 724

<sup>35</sup> Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs

Foodstuffs to which PAH limits apply include dried, salted or smoked fish, whether or not cooked before or during the smoking process. However Regulation 1881/2006 advises that when applying the maximum levels to foodstuffs which are dried or processed, the changes to the concentration of the contaminant caused by drying or processing must be taken into account. The essential question in relation to Katsuobushi is how this should be done. In relation to our referee case it was agreed that a factor supplied by the trader was acceptable to the competent authority. However, an estimate of the adjustment was also provided by one laboratory based on a moisture content determined on the sample and the generally accepted moisture content of the original fish fillet. We carried out calculations on the results supplied by each laboratory and additionally characterised the composition of the product by our own analysis. Multiple replicates of the referee sample were analysed for proximates (loss on drying, moisture, nitrogen, protein ( $N \times 6.25$ )<sup>36</sup>, lipids and mineral matter) by standard methods. In particular water was determined by the analysis of multiple replicates by coulometric oven Karl Fischer, KF, determination. The determinations were carried out in an automated microprocessor controlled instrument by transfer of water from around 10 milligrams of sample in a septum capped vial by controlled heating and dry nitrogen carrier gas to a KF titration cell with electrochemical endpoint. The method was validated by prior thermogravimetric analysis coupled to mass spectrometry, TGA-MS, and quality assured with a 0.1 % water in hydranal reference material. Although there is no agreed species average nitrogen content on a fat free basis for tuna a conservative factor of 3.97<sup>37</sup> resulted in an apparent total tuna content in the sample of over 300 %.

The trader asserted that the final product represented concentration factors of between 580 % and 680 %, data

that were accepted in this instance by the Food Standards Agency. This should not be a surprise, as the expected values of proximates in tuna are based on a fresh sample, where the water content is significantly higher than in these samples, which had been dried and smoked. In view of this we carried out a recalculation and concluded that appropriate treatment of the raw data produced by each of the laboratories that analysed the first and second portions of the formal sample demonstrated that in neither case is a non-compliance established beyond reasonable doubt against the limits for Benzo(a)pyrene and the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b) fluoranthene and chrysene respectively. That being so it was not necessary for the Government Chemist to establish independent referee data for the PAH in the sample. We also made a series of recommendations to the parties involved including advice to food business operators subject to official controls for PAH in a product that has been dried or concentrated on how they should supply a concentration factor that is supported with attested or audited mass balance raw data. We also asked for clarification on the basis for the limits for smoked fish in Regulation 1881/2006 in relation to concentration factors. We are now cooperating with the Food Standards Agency in drafting a paper exploring the issues raised in the case.

### Added water in fish, aluminium and aflatoxins

Lastly we explored with all the parties involved the issues in a dispute on the water content of fish fillets. As a result the dispute was resolved without recourse to analysis by the Government Chemist. The issues in the remaining two cases, those of aluminium in noodles and aflatoxins in pistachio powder and in nutmeg have been dealt with in previous annual reports.



<sup>36</sup> Nitrogen content is multiplied by a factor (in this case 6.25 for fish) to give the protein content

<sup>37</sup> Based on McCance & Widdowson, Composition of Foods, 5th Ed., supported by FAO data, <http://www.fao.org/wairdocs/tan/x5916e/x5916e01.htm>

# 3 Impact

The impact of the work of the Government Chemist programme is broad and the effects can be seen in a number of ways.

Research projects are carried out to support the future work of the Statutory Function and prioritised, for example, by horizon scanning to identify measurement topics which are likely to become more important. These projects have benefits beyond the referee analyses carried out by the Government Chemist, often having impact on the wider measurement community to prevent disputes by promoting best measurement practice in emerging areas of threat. Outputs are disseminated through knowledge transfer activities; a list of publications is given later in this section. The advisory function of the Government Chemist provides advice on analytical measurement subjects to Government, the European Commission, and the wider stakeholder community.

All these activities are aimed at predicting future regulatory issues within the chemical and biochemical measurements sphere, and providing a secure base for more efficient and cost-effective regulations.

## Horizon Scanning

Preparedness for future problems is enhanced by our horizon scanning of the scientific implications of policy development, emerging and changing legislation, and enforcement trends. We publish our foresight activities, such as our reviews on legislation with a commentary on the associated scientific context, on our website. We collaborate with IFST, Defra and the APA Training Committee, gaining and sharing insights on developments in the food industry and the official food and feed control system. An exciting novel aspect of our horizon scanning is our collaboration with Kingston University, co-funded by the Food Standards Agency, to enhance intelligence gained from multinational food

recalls datasets. This work explores the usefulness of interactive data mining of emerging or re-occurring temporal trends in global food safety and authenticity issues, and builds on the expertise and experience in Kingston University in the application of novel algorithms in Network Analysis coupled with web-based visualisation of outputs.





## Meat species identification – the use of Molecular Biology

### The issue

The current global economic position provides a more enticing backdrop for illegal activities in the adulteration of expensive foods using cheaper species and ingredients. The presence of undeclared species can have religious, ethical and economic repercussions for consumers, whose confidence in meat products was affected directly by the government findings earlier this year in relation to the horse meat scandal. This has led to heightened awareness of further meat contamination/adulteration that may be prevalent, including provision of meat produce intended for faith groups (e.g. pork in beef). The increased awareness of meat adulteration has mirrored increasing demands on the GC function as evidenced by 7 referee cases in the last year associated with meat species identification (horse, beef, pork and lamb) using DNA. The lack of traceability, via an audited paper trail, of some food ingredients and species, coupled with a deficit in the maintenance and development of reliable analytical tests to detect and quantify meat species of interest, has highlighted the need to develop and maintain effective systems to predict, monitor and test food chains for likely food and ingredient adulteration.

### The solution

We have developed methods using melt-curve analysis in real-time PCR to allow greater differentiation between target meat species - pork, beef, lamb, and poultry. In common with many kits and published literature, the meat speciation assays tested exhibited some cross-reactivity and were therefore not 100% specific. However, the addition of melt curve analysis provided valuable information for differentiation between species and provided a metric which could be used for comparative purposes. Using melt-curve analysis, selectivity was improved with all species, most noticeably for the detection of chicken and pork.

Novel PCR primers have been designed at LGC for a range of fish and meat species, including Vietnamese Catfish, cod, scampi, monkfish and kangaroo. These have been used to amplify DNA



from authenticated meat and fish samples. The related PCR products were sent off for DNA sequencing, and resulting data aligned with validated sequences on public databases to verify species identity. This has added to the repertoire of available molecular DNA methods the GC can employ as part of referee analysis, as well as providing valuable information on the use of DNA sequencing for species identity for food authenticity testing.

The FSAI published a report in January 2013 that stated that out of a total of 27 beef burger products analysed, 10 of these products (37%) tested positive for horse DNA and 23 (85%) tested positive for pig DNA. In one instance, when a beefburger on sale at a supermarket was analysed, results were reported that stated “the level of horse DNA indicated that horse meat accounted for approximately 29% relative to the beef content”. The Food Standards Agency (FSA) subsequently issued a four-point plan as part of the investigation: to conduct an urgent review of the traceability of the food products identified in the FSAI survey; to

explore the methodology used; to consider whether any legal action was appropriate; and to work with Defra on a UK-wide survey. Subsequently, it became clear that horse meat was a global issue involving illegal activity across geographic borders.

The GC assisted the UK Government on all aspects of the FSA four-point plan, working with FSA/Defra and providing advice on methods for determination of horse meat as part of the initial UK survey of beef products, as well as being members of the Project Steering Group. GC staff attended Government and industry led meetings as technical experts, and provided advice on the use of threshold levels for detection of horse meat, as well as assessing methods and advising on these for their applicability in the determination of horse meat. Advice was also provided to Public Analysts regarding best measurement practice advice when applying kits and methods for meat species determination in a laboratory setting. Separately, advice was provided to FSA on methods for the determination of phenylbutazone ('bute').



LGC also led on Defra projects to establish the Limits of Detection of methods used in the UK survey of beef products for horsemeat, and to help develop a real-time PCR approach for quantitation of horse DNA. The GC has representation on Defra's Authenticity Methods Working Group (AMWG) and associated DNA sub-group, and has provided advice on modern molecular approaches for meat analyses. Additionally, GC staff attended an EU consultative meeting in Brussels in September 2013, accompanying the FSA as the nominated UK expert laboratory on horse meat testing, and provided best measurement practice advice on molecular biology approaches.

Work has also been carried out on the development of methods for meat speciation, to evaluate and optimise methods for DNA extraction and sample preparation, to provide reviews on the impact of food processing on meat analyses, and a report on available approaches for meat quantitation. This was put to good use on a number of referee cases, which are covered elsewhere in this Review.



### Impact of the GC work on meat analysis:

The improved and standardised DNA approaches for meat analysis will facilitate:

- Improvements in methodology used for referee analysis, including shorter analysis times, lower limits of detection and more accurate quantitation.
- Improvements in regulation and enforcement including better product labelling, increased traceability of food products and components internationally, and the ability to test verifiable authenticity claims.
- Benefits to trade and industry including dissemination of knowledge and best measurement practice, helping honest traders defend label claims and the avoidance of costly food recalls.



## Chromium speciation – still a hot topic for many laboratories

### The Issue

Chromium (Cr) is a widely spread element that has been used by humans for more than 2000 years. It was first described in 1797 and since has been used in a wide range of applications affecting everyday life, from providing a corrosion-resistant coating to a main component in dyes and preservatives, and an essential element in food supplementation. It is well known that chromium bioavailability and toxicity varies widely with its chemical form. Chromium typically exists in one of two oxidation states: Cr(III), or trivalent chromium, and Cr(VI), or hexavalent chromium. While Cr(III) is an essential trace element involved in lipid and glucose metabolism, high concentrations and chronic exposure to it results in weight loss, anaemia, liver dysfunction, renal failure and other possible negative health effects. Cr(VI) compounds are known to be mutagenic and to affect the respiratory, cardiovascular, gastrointestinal, hepatic, renal, and neurological systems. For these reasons, Cr(VI) is considered toxic at any concentration level. Unfortunately due to its chemical properties, in nature chromium, in form of trivalent chromium can be transformed to hexavalent chromium and vice versa, which means that Cr health effect can change from essential to toxic at the same concentration! This fact is well recognised by regulatory organisations and Cr is monitored and regulated in many sample types, including environmental, food and feed additives, drinking water, pharmaceutical and consumer products. For example, Cr is regulated in drinking water at a maximum allowable level of around 50 to 100  $\mu\text{g L}^{-1}$ , but for the hexavalent form much more stringent guidelines and regulations exist down to the level of 0.02  $\mu\text{g L}^{-1}$ . The average Cr burden for UK adults is estimated to be around 150  $\mu\text{g day}^{-1}$  with about 117  $\mu\text{g day}^{-1}$  coming from food, 10  $\mu\text{g day}^{-1}$  from water (2 L  $\text{day}^{-1}$  drinking basis) and the remaining from other sources. In food and feed additives chromium is expected to be (mostly) in the form of Cr(III), while in water Cr(VI) is frequently found. Cr(VI) is unstable inside the human body and rapidly reduced to Cr(V), Cr(IV) and finally to Cr(III) but there is strong evidence that in form of Cr(VI) it can enter cells

(through sulphate and phosphate channels) and exhibit strong toxicological effects.

Therefore to increase public confidence in the quality of the UK environment and drinking water, in addition to the total Cr concentration, reliable speciation methods are needed.

The state of the art techniques, which provide accurate and precise chromium speciation information, require availability of high-end analytical instrumentation, expensive isotopically enriched materials (to perform isotope dilution analysis) and specially trained personnel to perform the analysis. For this reason, in 2013, the Government Chemist Working Group (GCWG) initiated a project to develop a method for the quantitation of chromium (III) and (VI) in clean and effluent water. The project aimed at developing a simple and straightforward methodology for the determination of inorganic Cr species in water using conditions that minimise species transformation, and that can be easily implemented in field laboratories. It also included the validation of this methodology by comparison with results obtained using more tedious, high accuracy methods such as those involving species-specific isotope dilution calibration.

### The solution:

A method based on ion pair/chelation reversed phase High Performance Liquid Chromatography Inductively Coupled Plasma Mass Spectrometry (HPLC-ICP-MS) for the quantification of both Cr(VI) and Cr(III) species in water was developed. The method relies on external calibration and does not require isotopically enriched materials. Since at the time of the study, no water CRM certified for Cr species (trivalent and hexavalent chromium) was available, a set of AQUACHECK proficiency testing scheme solutions (clean and waste waters, supplied by LGC) were analysed to evaluate the trueness and applicability of the method. The external calibration results were compared to those obtained with the isotope dilution method. An estimation of the limits of detection for the external calibration method was made and spike recovery

experiments were performed. The same analytical column and stationary phase was used for both the external calibration and SS-IDMS analyses. The chromatographic conditions, the ICP-MS conditions and the specific chromium isotopes measured were optimised. This method, which has been published on the Government Chemist website<sup>38</sup>, allows 3 samples per hour to be analysed for chromium species. The instrumental limit of detection for Cr(VI) was 0.02  $\mu\text{g L}^{-1}$  which is adequate for the expected levels of Cr in drinking water. The method is fast and simple and the only sample preparation required is dilution with a concentrated form of the chromatographic eluent. The agreement between the assigned AQUACHECK PT-values and experimental chromium speciation values was within 6 % (Recovery 97 to 103 %). The hexavalent chromium spikes recoveries for clean and waste water spiked PT-samples varied between 102 % and 111 %. These results are within the acceptable range and are in good agreement with the assigned values, illustrating the trueness of the method.

Real samples were analysed by the external calibration isotope method. The isotope dilution method has the advantage of accounting for species inter-conversion being Cr(III) oxidizing to Cr(VI) in presence of oxygen and/or basic media (pH>8), or Cr(VI) reducing to the lower oxidation state in presence of organic compounds or acidic matrix (pH <5). Waste water PT-samples, both natural and spiked were analysed by both methods. The results compared very well between these methods, to within 1%, which validated the external calibration method.. It is therefore suitable for routine use for the determination of both Cr(VI) and Cr(III) species in clean and effluent waters. Laboratories, if equipped with suitable ICP-MS and HPLC instrumentation, will be able to use this procedure, providing they monitor the common Cr interferences, and take account of them. The sample preparation is simple and cost-savings are realised by using inexpensive natural abundance calibration standards.

<sup>38</sup> <http://www.governmentchemist.org.uk/Generic.aspx?m=78&amid=1739>

## Food Allergy – a call to action

### The Issue:

Food allergy, now a well recognised public health challenge, is an adverse reaction mediated by the immune system to proteins that are harmless to most of the population. Adverse consequences for those affected include detriment to their quality of life, risk of death by anaphylaxis and problems for businesses in ensuring allergen-free products. Although prevalence data is patchy over 1.5 million adults and children in the UK may have a diagnosed food allergy and recalls of food stemming from mistakes with allergen formulation, labelling or cross contamination could be costing UK businesses up to £80M annually.

What has this to do with measurement science? The risk assessment, management and regulation of allergens in food depend in large part on our ability to detect and measure them. The methods currently available for measuring allergens in food are not optimal. For example the most commonly applied approach that depends on manufactured kits containing antibodies, exhibits manufacturer-specific and variable sensitivities and cross-reactivity. Structural changes by food processing or sample extraction may prevent detection at all and impact adversely even on more modern and high technology methods.

Recent developments are close to providing significant advances in allergen risk management through robust evidence for clinically derived thresholds at which defined proportions of the allergic populations react. Legislation imposing more stringent allergen labelling will bite in 2014. Oral immunotherapy for allergy is emerging as a potential treatment. Good measurement underpins all this yet there are no suitable reference standards available for allergen measurements which are internationally traceable.

This work provides an opportunity for the international metrology community to use these developments to make a meaningful difference.

### What is food allergy?

Food allergy is a pathological immunological reaction in sensitised individuals to food proteins that are harmless to the majority of the population. Mediated by an immunoglobulin, IgE, food allergy is a well recognised public health challenge. Adverse consequences include detriment to the quality of life for allergic consumers, risks of fatalities by anaphylaxis and problems for businesses. About 1-2% of adults and 5-8% of children may have a diagnosed food allergy, equating to up to 800,000 children and a million adults in the UK. Prior to horsemeat, the largest single cause of food recalls in the UK involved allergens, potentially costing up to £80m annually. Much has been learned about the complex biochemistry and immunology of allergy but this has so far not translated into fundamental explanations of why the condition is increasing in industrialised populations nor has it resulted in prevention or cure. The only current therapeutic approach for those with diagnosed food allergy is avoidance of the problem food although oral immunotherapy shows some promise. Food law, particularly labelling of allergens, has grown in proportion to the problem with, in December 2014, new obligations on catering businesses to provide information on the 14 major allergens.





## Measurement science towards traceable allergens methods

The Government Chemist has led the way in pioneering a model for the absolute quantification of allergenic protein in food in a traceable manner so that measurements made at different laboratories across the globe can be compared in a meaningful way. The model applied proteolytic digestion of the allergen and isotope dilution mass spectrometry. Applying these costly techniques centrally facilitates development of quality control and reference materials economically to improve allergen analysis to suit every pocket. But there are dozens of major allergen proteins and many food matrices – it will take a globally coordinated effort to address these.

We collaborated with key stakeholders in the UK, Ireland and internationally to improve immunological analysis of allergens. Led by the University of Manchester, a multi-laboratory evaluation of a clinically-validated quality control material for analysis of allergens in food, involving partners as far afield as Japan and Australia took place. Stemming from this we are in the process of developing the first clinically associated incurred peanut allergen quality control material in a realistically challenging food matrix. More work of this nature is needed. For a number of reasons the power of DNA analysis has not had as much application to allergen detection as perhaps it should.

Lastly, in cooperation with a patient support group, we reviewed court cases dealing with incidents of anaphylaxis resulting in death or serious injury. With a better understanding of the attitude of the courts to these, often preventable, tragedies we aim to develop guidance on sampling, analysis and interpretation in serious anaphylaxis incidents.

Thus in all the key allergen measurement disciplines we are making advances, working to understand the context better and provide tools to assist the analytical and allergen communities.

### LC-MS

Isotope dilution mass spectrometry is the fundamental underpinning technique for traceable protein quantitation measurements according to the International System of Units (the SI). The need for this in the case of allergen protein measurement was recognised by the Government Chemist and in 2013 the culmination of a collaboration between the GC Programme and the NMI was published. The study on exact matching isotope dilution mass spectrometry absolute quantification of the egg allergen protein lysozyme in wine was, to our knowledge, the first demonstration of such work. We have gone on to apply the expertise developed to the selection, synthesis and characterization of suitable peptides for the analysis of  $\alpha$  S1-casein, a significant milk allergen protein. The peptides, monitored on our newly-acquired triple quadrupole MS, proved stable during food processing and led to successful quantification of casein in aqueous solution by trypsin digestion and LC-MS/MS. Promising results were obtained when the method was applied to determining the extraction efficiencies of casein from dairy free biscuits spiked with casein. It was also used to explore the recovery of  $\alpha$  S1-casein fraction by ELISA, described below.

### ELISA

Work carried out by the University of Manchester Allergy Network suggests that current ELISAs can determine the presence, but not quantify accurately, the levels of allergens in foods.

Integration of MS with ELISA applied to milk allergens has also been a theme of our own work. Along with egg, milk is the most prevalent of the childhood allergies and the ubiquity of skimmed milk powder as a functional ingredient in the food industry makes this a key analytical target. Looking specifically at the  $\alpha$  S1-casein fraction of milk protein we compared available ELISA kits for casein and with an LC-MS/MS approach. Experiments on  $\alpha$  S1-casein spiked into unbaked and baked biscuits yielded interesting results on the recovery of  $\alpha$  S1-casein fraction by three ELISA kits. An abstract describing the work was presented to the 'Recent Advances in Food Analysis', RAFA, conference in Prague in November 2013.





### DNA

For a number of reasons the power of DNA analysis has not had as much application to allergen detection as perhaps it should. However, DNA approaches for the detection of specific allergenic species offer potential advantages to their protein based counter parts including improved method performance characteristics of selectivity and sensitivity, as well as detecting some allergenic species that protein based techniques cannot (e.g. specific nuts, cereal varieties containing gluten and molluscs). We have developed powerful DNA screening approaches for the detection of allergenic food ingredients with assays for the identification of peanut and additional tree-nut species including almond and hazelnut. We investigated the feasibility of DNA assays for allergenic ingredients such as soya, celery, sesame seed and mustard on "lab-on-a-chip" capillary electrophoresis systems. Spin-off from such approaches includes "point of test" applications, the potential to multiplex assays for economies of scale, and the application of such DNA tests across a range of common laboratory instrumentation to increase the scope and usefulness of the methods

## Knowledge Transfer

The Government Chemist seeks to benefit innovation and regulation by dissemination of knowledge gained through our work particularly in referee analysis. Such dissemination is aimed mainly to improve knowledge and skills in the analytical community through a coherent package of knowledge transfer activity which includes

- the publication of case studies based on actual referee analysis;
- the organisation of training in collaboration with the APA Educational Trust and the Food Standards Agency;
- proactive input to key stakeholder organisations and
- provision of sound advice to stakeholders.

## Advice

We continued to respond to requests for advice from a wide range of stakeholders. Many concern disputed analytical results but were resolved by advice rather than referee cases. This year's advice covered topics as diverse as food dyes, allergens and the arrangements for the referee analyst under the Feed (Sampling and Analysis and Specified Undesirable Substances) Regulations (Northern Ireland) 2010.

There were intensive calls on our expertise by the media during the first six months of the horse meat episode with live and recorded interviews in national broadcast media and articles in the print media. This led to a developing relationship with the Science Media Centre which seeks to ensure that the public have access to the best science through the news media when science hits the headlines. In all our media work we presented the science in a balanced and responsible manner and found that the journalists we worked with wanted nothing less, helping to promote a rational and well informed debate.

## Training

The Government Chemist acquires a great deal of expertise and knowledge through discharging the statutory function. This forms the basis of material which can be used in the provision of training for practising analysts.

In September, Malcolm Burns, Principal Scientist and Special Adviser to the Government Chemist, organised a Knowledge Transfer (KT) event for Public Analysts and industry aimed at designing PCR primers to help with food authenticity testing. The KT event was jointly funded by the Department for Environment, Food and Rural Affairs (Defra) and the FSA and was fully subscribed with attendance from 15 UK Public Analysts. The KT event was a direct follow up to a previous one held at LGC in early 2011, which equipped Public Analysts with the knowledge and tool set to confidently utilise DNA sequencing for a range of

animal/plant species for which there were pre-existing FSA/Defra protocols. Since the original course LGC and Defra have received further requests from Public Analysts asking for guidance on how to sequence DNA from a range of new and emerging species not included in pre-existing FSA/Defra protocols.

In cooperation with the APA Educational Trust, our joint flagship training activity, the intensive annual week long course held at Reading University was again a success. In April / May 2013 we hosted 14 delegates, the majority from Public Analyst Laboratories, the teaching consisting of a mix of lectures, laboratory practical sessions and interactive exercises. We are again grateful to the lecturers who present a wealth of technical and interpretative detail in a well prepared and authoritative manner. The Public Analysts' laboratories represented some 250 local authorities in the UK and Isle of Man and both delegates and speakers spanned both the private and public sector laboratories that make up the modern Public Analyst service. Further detail is available on the GC website and a short report on the course was sent to FSA and appeared in FSA eNews page 4: <http://www.food.gov.uk/multimedia/pdfs/publication/fsa-e-news-may2013-vs2.pdf>

We also made the highly regarded LGC courses on measurement uncertainty available to public analysts who were also happy to take part in our allergen webinars.

## The Wider Advisory Function

The Government Chemist also has a role to provide advice on subjects with analytical measurement aspects to both Government (including the European Union and devolved administrations) and the wider community of stakeholders, which includes industry, academe and local Government. This is done by means of the provision of specific advice pertaining to aspects of measurement topics on a broad range of policy and regulatory developments, and also providing a proactive scientific and measurement-based support service to those industries where chemical measurements are key to their business.

## Addressing scientific issues with stakeholders

We have continued to follow developments of both the UK Chemical Stakeholder Forum and the Hazardous Substances Advisory Committee (HSAC), the successor body to the Advisory Committee on Hazardous Substances, by attending meetings of these bodies and, where appropriate, making contributions to relevant discussions. We have become the *de facto* experts on analytical measurement issues within the HSAC, and have been frequently asked to provide an opinion on this where required. A good example of this was in the provision of advice to the Committee on the analytical methodologies pertinent to the determination of polynuclear aromatic hydrocarbons (PAHs) in toys and childcare products in response to a proposal under REACH<sup>39</sup> to impose significantly lower limits on the compounds. This involved explaining the difference between total PAHs and available PAHs, and the methods used to determine these.

We are also members of the Steering Committee of the Standing Committee of Analysts (SCA) representing the Government Chemist. The SCA, sponsored by the Environment Agency, comprises a series of working groups who provide authoritative guidance on methods of sampling and analysis for determining the quality of environmental matrices. Guidance is published as Blue Books within the series Methods for the Examination of Waters and Associated materials.

We have continued to provide advice through our responses to a wide range of official consultations (see Box 2). These consultations are carried out by the Government (including devolved administrations and agencies), Standards bodies or the European Union, to obtain the input of both interested and expert stakeholders on proposed new legislation or regulations, prior to enactment and are considered by legislators to be an

important part of the development process for new legislation and regulation. The Government Chemist is well-placed, through the expertise within LGC in a breadth of matters in analytical science, to respond authoritatively and independently to a wide range of consultations which have chemical or bioanalytical measurement implications.

### Box 2. Our Public Consultation Responses

Department for Environment, Food and Rural Affairs	Food Information Regulations 2013
Health & Safety Executive	Consultation on regulatory measures to support EU chemicals legislation and proposals on reducing seven existing sets of domestic regulations into one Statutory Instrument (7 into 1 package)
Food Standards Agency	Radioactivity in Food Monitoring Review
Department of Business, Innovation and Science	Enforcement of Cosmetic Regulations 2013
Food Standards Agency (Scotland)	The Contaminants in Food (Scotland) Regulations 2013
National Measurement Office	Consultation on OIML review of Recommendation 100 Atomic absorption spectrometer systems for measuring metal pollutants in water – Final Draft
Food Standards Agency	The Approval of Establishments Producing and Processing Certain Fats and Oils, and the Testing of Fats and Oils of Vegetable and Marine Origin for Dioxins and Dioxin-Like PCBs.*
Department for Environment, Food and Rural Affairs	Review into the Integrity and Assurance of Food Supply Networks – Call for Evidence
House of Commons Energy & Climate Change Select Committee	Written Submission on Carbon Capture and Storage (CCS)
Department of Business, Innovation and Science	Proposals for two new European regulations aimed at improving consumer product safety and the functioning of the European internal market through effective market surveillance.
Department for Transport	Regulations to specify the drugs and corresponding limits for the new offence of driving with a specified controlled drug in the body above the specified limit
Food Standards Agency	The Feed Law Code of Practice Review
European Commission DG Environment	Public Consultation on the revision of the Annexes of the Groundwater Directive
Food Standards Agency	Proposed changes to Official Controls

\* This consultation response was also sent to the appropriate department within the devolved administrations in Wales, Scotland and Northern Ireland

<sup>39</sup> Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals, as amended

#### Specific questions which we addressed included:

- The concept of risk versus hazard, and the role of analytical methodology in explaining this
- The need to ensure that analytical measurement techniques are fit-for-purpose in the enforcement of proposed legislation concerning driving whilst under the influence of drugs
- The importance of considering the chemical contamination aspects in carbon dioxide transport and storage in carbon capture and sequestration
- The importance of appropriate and accurate statistical processes in properly interpreting analytical measurement data
- The need to ensure that sufficient samples are taken in the event of a possible dispute which could result in a referee case carried out by the Government Chemist
- The use of existing quality standards, particularly ISO/IEC 17025, as a valid means of identifying a laboratory's competence.

## Dissemination

During 2013 we continued to promote the Government Chemist blog<sup>40</sup> which we use to communicate stories and issues where legislation and regulation meet analytical measurements. The blog is aimed to be informal and a means to make stakeholders aware of a range of issues of interest.

<sup>40</sup> <http://governmentchemist.wordpress.com/>

## Taking our advice into new areas

We sought the opinion of stakeholders to determine which sectors would benefit from support in the understanding and implementation of issues within important legislation such as the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and the Water Framework Directive (WFD). As a consequence we identified the oil sector, based in North-East Scotland as an area where there is scope for increasing knowledge in new regulations.

As a result we organised a seminar on issues where regulation and measurement overlap within this important sector. This was held at the Thistle Hotel, Aberdeen Airport and was well attended with 40 delegates, many of whom were from the oil industry in Scotland.

#### The talks were of a high quality and covered the various issues from different perspectives:

- Regulatory Overview (DECC)
- WFD and PHS Legislation (SEPA)
- REACH Enforcement and how it applies to the Oil Industry (HSE)
- Offshore Environmental Compliance – The Measurement Challenge (Intertek)
- Innovation in Flow Measurement in the Oil and Gas Industry (TUV-NEL)

Full details of all the presentations can be found on the Government Chemist website ([www.governmentchemist.org.uk](http://www.governmentchemist.org.uk)).

## Micro-funded Studies

The prioritisation process undertaken by the GCWG prior to the commencement of the 2011-2014 programme identified a number of proposed project areas which they felt were appropriate for small-scale funding.

#### Studies which were concluded in the last year were:

- The quantitative determination of chromium species in clean and effluent waters by LC-ICP-MS. This work is explained in more detail elsewhere in this review (p20). It is potentially transferrable to other matrices if the extraction and clean-up can be successfully addressed, and a further project may be undertaken during 2014 to look at this. One potential application concerns being able to measure chromium (VI) in leather belts, which is the subject of a REACH restriction.
- Physicochemical characterisation of silica nanoparticles in complex food matrices. This work was carried out under supervision at LGC by an MSc student from Loughborough University, and concentrated on the verification of methods which could be used in support of the European Commission's proposed definition of a nanoparticle. The report is available on the Government Chemist website.

## Chemical Nomenclature

Substance identity is very important in attaining compliance with legislation, particularly relating to chemical safety. REACH requires accurate naming of chemicals so that correct procedures can be followed to use chemicals safely, or to deal with problems efficiently if they occur.

It is also important to use correct names in publications to aid communication. A paper reporting high-class research can be rendered worthless if it is not clear which chemicals are involved. Kevin Thurlow has been a member of IUPAC's Advisory Committee to Chemical Nomenclature and Structure Representation Division (VIII) since its inception in 2002. He has also represented the Government Chemist on RSC's "Committee on Standards in Nomenclature, Terminology, Units and Symbols" (CSN) since 1991.

The former committee is "virtual". Members are invited to comment on draft proposals and documents and to participate in drafting of new or revised recommendations for chemical nomenclature.

The RSC committee meets once a year. Most members are concerned with education (higher and further), but there are also representatives of BSI and scientific societies on the committee.

The new IUPAC book of recommendations on construction of the Preferred IUPAC Name (PIN) for organic compounds has now been published. It has taken some years to reach agreement on the recommendations and the book is over 1500 pages long. Our comments were taken into account before publication.

The LGC Forensic Drugs team assists the Department of Justice in the preparation of amendments to legislation. Our input to this was in the supply of accurate chemical names and descriptions so that legislation could deal with "legal highs". It is important that the correct chemicals or families of chemicals are banned, whilst

allowing harmless chemicals, or legitimate medicines to be items of trade. Many of the "legal highs" are chemically very similar to legitimate products, so care must be taken in drafting technical aspects of legislation to ensure structurally similar but harmless compounds are not inadvertently caught by the law.





# Publications

Publishing peer-reviewed papers is integral to our work enabling transparency to the analytical community. The following were published in 2013:

Burns M, Nixon G, Walker M & Busby E, Development of an in-house Plasmid Control for Cauliflower Mosaic Virus (CaMV) for the Detection of Genetically Modified (GM) Chinese Rice Lines, JAPA (Online), 2013, 41, 53-66

Cryar A, Pritchard C, Burkitt W, O'Connor G, Walker M, Thorburn Burns D and Quaglia M, Towards Absolute Quantification of Allergenic Proteins in Food—Lysozyme in Wine as a Model System for Metrologically Traceable Mass Spectrometric Methods and Certified Reference Materials, JAOAC International, 2013, 96, 6, 1350-1361

Dunn P, Malinovskiy D & Goenaga-Infante H, Determination of absolute carbon isotope ratios in a glycine candidate reference material using EA-IRMS and different calibration approaches, Rapid Communications in Mass Spectrometry, RCM-13-0139

Gutteridge A & Burns M, The Application of DNA Molecular Approaches for the Identification of Herbal Medicinal Products, JAPA (Online), 2013, 41, 45-52

Malinovskiy D, Dunn, P & Goenaga-Infante H, Determination of absolute  $^{13}\text{C}/^{12}\text{C}$  isotope amount ratio measurements by MC-ICPMS using calibration with synthetic isotope mixtures, Journal of Analytical Atomic Spectrometry, 2013, 28, 1760-1771

Pritchard C, O'Connor G & Ashcroft A, The role of ion mobility spectrometry-mass spectrometry in the analysis of protein reference standards, Anal Chem (Online) 10.1021/ac400927s; 2013, 85(15), 7205-7212

Taylor A, Day MP, Hill S, Marshall J, Patriarca M & White M, Atomic spectrometry update. Clinical and biological materials, foods and beverages, Journal of Analytical Atomic Spectroscopy, 2013, 28, 425-459

Urgast DS, Hill S, Kwun I-S, Beattie JH, Goenaga-Infante H & Feldmann J, Zinc isotope ratio imaging of rat brain thin sections from stable isotope tracer studies by LA-MC-ICP-MS, Metallomics, 2012, 4, 1057-1063

Walker M, Woolfe M & Gurung T, Can Analytical Chemists do Molecular Biology? A Survey of the Up skilling of the UK Official Food Control System in DNA Food Authenticity Techniques, Journal of Food Control, Food Control 33, 385-392 DOI information: 10.1016/j.foodcont.2013.03.015

Walker M & Axford I, Allergy Action Plan, Chemistry & Industry, May 2013

Walker M & Gray K, Quis custodiet – a Review of the Resolution of Disputed Chemical Results in the UK Official Feed and Food Control System 2010 – 2011, JAPA (Online), 2013, 41, 1-27

# Glossary

See the International Vocabulary of Metrology<sup>38</sup> for the current definitions of terms used in measurement science

<b>ALARA</b>	As low as reasonably achievable
<b>AMWG</b>	Authenticity of Methods Working Group (Defra)
<b>APA</b>	Association of Public Analysts
<b>BSI</b>	Department for Business, Innovation and Skills
<b>Defra</b>	Department for Environment, Food and Rural Affairs
<b>Derivatisation</b>	Chemical modification of a substance, typically without changing its core structure, for example to facilitate measurement
<b>DfT</b>	Department for Transport
<b>DH</b>	Department of Health
<b>DNA</b>	Deoxyribonucleic acid
<b>ECHA</b>	European Chemicals Agency
<b>EFSA</b>	European Food Safety Authority
<b>ELISA</b>	Enzyme-linked Immunosorbent Assay
<b>EU-RL</b>	European Union Reference Laboratory
<b>FBO</b>	Food or feed business operator
<b>FSA</b>	Food Standards Agency
<b>FSAI</b>	Food Safety Authority of Ireland
<b>FCM</b>	Food Contact Material

<b>GC-MS/MS</b>	Gas chromatography-tandem mass spectrometry
<b>GCWG</b>	Government Chemist Working Group
<b>HPLC-ICP-MS</b>	High performance liquid chromatography linked with inductively coupled plasma mass spectrometry
<b>HSAC</b>	Hazardous Substances Advisory Committee. Expert committee providing advice to Government on hazardous substances, toxicology, risk assessments.
<b>IDMS</b>	Isotope dilution mass spectrometry; a technique capable of outstanding accuracy
<b>IFST</b>	Institute of Food Science and Technology
<b>IUPAC</b>	International Union of Pure and Applied Chemistry
<b>JAOAC</b>	Journal of the Association of Official Analytical Chemists. A leading international journal for analytical measurement topics supporting legislation.
<b>KF</b>	Karl Fischer, an analytical technique for measuring water at low levels
<b>LC-MS/MS</b>	Liquid chromatography-tandem mass spectrometry
<b>LOD</b>	Limit of Detection
<b>MChemA</b>	Mastership in Chemical Analysis – this Royal Society of Chemistry qualification is required for appointment as a Public Analyst or as an Official Food Analyst

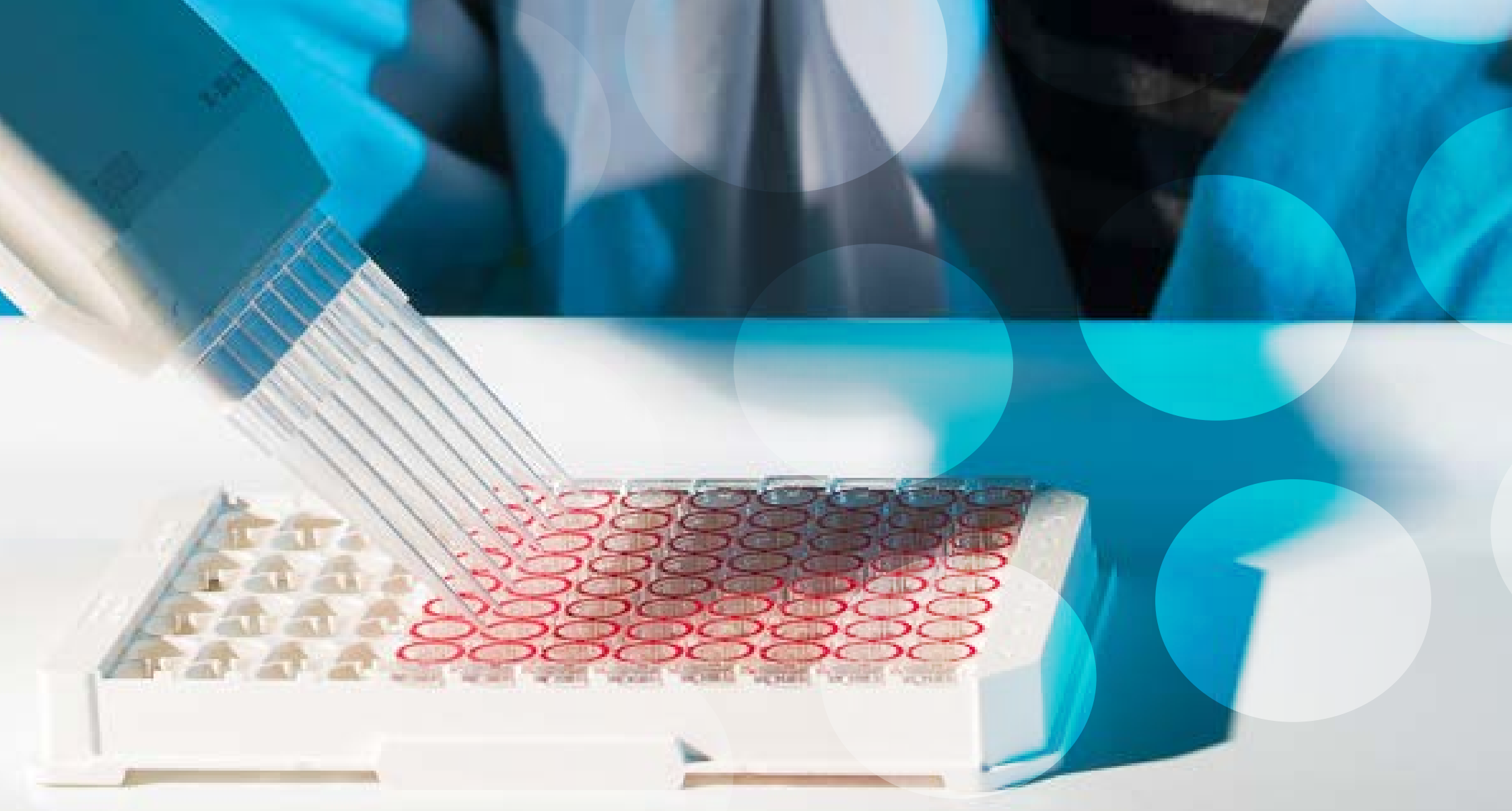
<sup>38</sup> International Bureau of Weights and Measures, International vocabulary of metrology – basic and general concepts and associated terms (VIM), Third Edition, JCGM 200:2008, 2008, [www.bipm.org/utis/common/documents/jcgm/JCGM\\_200\\_2008.pdf](http://www.bipm.org/utis/common/documents/jcgm/JCGM_200_2008.pdf)

# Glossary

<b>MRL</b>	Maximum recommended limit
<b>NMI</b>	National Measurement Institute
<b>NMO</b>	National Measurement Office
<b>OIML</b>	Organisation Internationale de Métrologie Légale (International Organization of Legal Metrology)
<b>Official Food Analyst</b>	A person qualified under the Food Safety (Sampling and Qualifications) Regulations (1990 and/or 2013) (see also MChemA and Public Analyst)
<b>PAH</b>	Polynuclear aromatic hydrocarbon, a group of toxic chemicals
<b>PCR</b>	Polymerase chain reaction, a technique used to amplify DNA sequences so that they can be identified
<b>Port Health Authority</b>	Special type of local authority created to ease administration at seaports where the port area is covered by more than one local authority, responsible for carrying out checks on food and feed consignments
<b>Public Analyst</b>	Analytical scientist appointed under statute by UK local authorities to provide an official food or feed control function and scientific advice for the enforcement of many acts of Parliament
<b>Quantitative analysis</b>	Measurement, with results expressed as a number and a unit, of the quantity of a target substance in a sample, e.g. 10 mg.kg <sup>-1</sup>
<b>RASFF</b>	EU Rapid Alert System for Food and Feed
<b>REACH</b>	Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals, as amended

<b>Referee analysis</b>	Impartial analysis by the GC to help resolve disputes relating to test results obtained on behalf of two independent parties
<b>Referee function</b>	Duty of the Government Chemist under acts of Parliament to provide impartial analysis in the resolution of disputes relating to the enforcement of regulation
<b>SEO</b>	Supplementary expert opinion in the context of Regulation (EC) No 882/2004 on official controls, Article 11(5)
<b>SS-IDMS</b>	Species-specific isotope dilution mass spectrometry
<b>Tandem mass spectrometry</b>	use of linked mass spectrometers; molecules of interest can be broken up after the first stage to allow more detailed characterisation by analysing their fragments in the second
<b>TGA-MS</b>	Thermogravimetric analysis linked to mass spectrometry
<b>UKCSF</b>	United Kingdom Chemical Stakeholders Forum
<b>UKAS</b>	United Kingdom Accreditation Service
<b>WFD</b>	European Union Water Framework Directive





Queens Road, Teddington, Middlesex TW11 0LY, UK  
Tel: +44 (0)20 8943 7000 Fax: +44 (0)20 8943 2767  
[www.lgcgroup.com](http://www.lgcgroup.com)

All external links operational on 03 April 2014.  
Subject to Crown licence. No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording or any retrieval system, without the written permission of the copyright holder. © LGC Limited, 2014. All rights reserved.

