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Walker *et al*

Quis custodiet – a Review of the Resolution of Disputed Chemical Results in the UK Official Feed and Food Control System 2010 – 2011

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This paper is based on a presentation made at the 2012 Government Chemist Conference – Safe Authentic Food, Policy & Enforcement Challenges & Opportunities held on 8-9 May 2012 at the Royal Society, London.

Summary

EU Regulation 882/2004 harmonises official controls on feed and food and provides for businesses subject to those controls to have an analytical second opinion. UK law predates this safeguard by over 100 years. The regulatory landscape in the UK is a complex one with policy set by central government and enforcement mainly a local government responsibility. Analysis by official food and feed control laboratories "the OCL system" provides the underpinning measurement science. Chemical aspects of food and feed safety, composition and labelling are dealt with by Public Analysts and Agricultural Analysts (generally the same official). Should an analytical dispute arise a retained portion of the control sample may, in statutorily defined circumstances, be submitted as a technical appeal to the Government Chemist for a definitive investigation, the "referee analysis".

This paper describes Government Chemist referee casework in the calendar years 2010 and 2011 and provides an opportunity to assess the performance of the technical appeal safeguard and the control system in the limited number of complex cases where appeal has been invoked.

The OCL system in the UK faced continuing funding challenges in 2010 and 2011 but in general performed well in areas where capability has been developed such as in aflatoxin analysis where Public Analysts' and Agricultural Analysts' findings were confirmed on technical appeal in 5 out of 7 (71.4 %) cases. However much more dispersion is evident in aflatoxin results between laboratories in animal feed samples than in food samples. Since largely the same laboratories are involved it is clear that sampling, and in particular the lack of a requirement for high shear mixing with water to form a slurry prior to splitting the samples into parts, is the main source of the variation. It is recommended that sampling and sample preparation should be harmonised in the feed and food areas.

OCL performance was less good in the more problematic area of drug residue analysis. Of six nitrofuran marker metabolite cases (all on imported crustaceans) only one (17 %) was completely upheld. In 3 cases (50 %) a residue was confirmed present but at a concentration below the limit at which the consignment should be prohibited from entry into the UK.

Research published in 2011 demonstrated that the marker for nitrofurazone, semicarbazide SEM, is naturally occurring in crustacean shells and in two cases (33 %) the Government Chemist confirmed SEM not detected in the core flesh of the animals overturning the OCL findings. We published a comprehensive advice note on nitrofuran analysis and since then the number of disputes has diminished with only one in 2012 in which the OCL findings were upheld. It is recommended that proposed better markers for nitrofurazone, such as its cyano derivative, should be further investigated and that the sampling of imported crustaceans should be reviewed to achieve better homogenisation prior to sample subdivision.

The diversity of measurement methods surveyed in the paper is evident and indeed represents only a fraction of the range of techniques OCL's and the Government Chemist must competently deploy to ensure the system is effective and responsive.

In animal feed analysis issues persist around tolerances, vitamin determinations and interpretation of Regulation (EC) No. 767/2009 and will be the topics of a separate case study paper.

Introduction

Sed quis custodiet ipsos custodes?[1]

Food and animal feed must be safe, authentic and properly labelled, responsibility for which falls to those who make and sell it[2]. There is, however, a public expectation of regulatory oversight by government and although thinking in this area continues to evolve[3] EU Regulation 882/2004[4] harmonises official controls on feed and food.

The regulatory landscape in the UK is a complex one with policy set by central government and enforcement mainly a local government responsibility[5]. Sampling and analysis are important features of enforcement official controls. In the UK, official food and feed control laboratories[6] (the OCL system) provide the underpinning measurement science in a range of laboratory facilities; the chemical aspects of food and feed safety, composition and labelling are dealt with by Public Analysts and Agricultural Analysts (generally the same person). The responsibilities of Public Analysts also, particularly in Scotland, include acting as Food Examiners dealing with microbiological safety and quality. Public Analysts and Agricultural Analysts are represented by the Association of Public Analysts, APA[7]. Other microbiological laboratories also undertake Food Examiner microbiological work for local authorities[8].

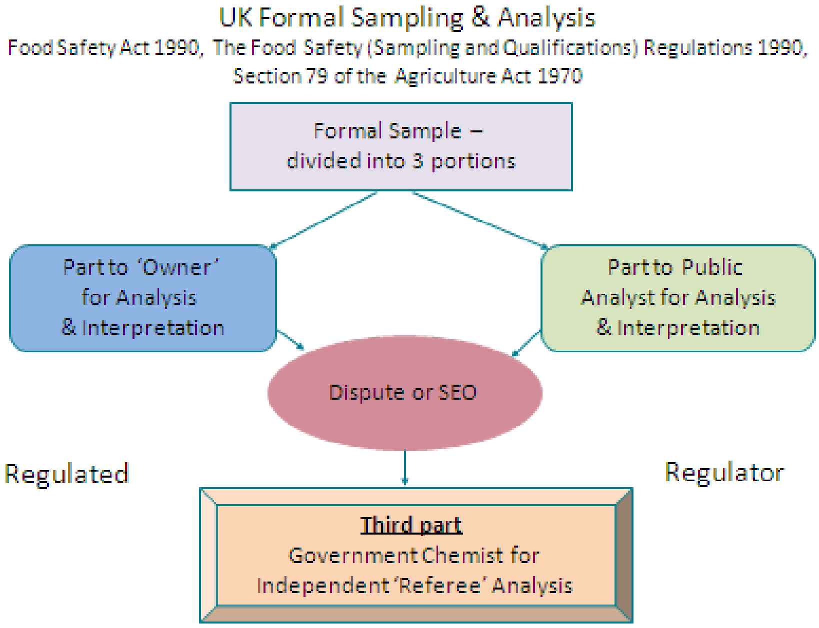
Since the inception in the 19th century of regulation of food and feed, UK law has provided safeguards. The formal control sample is split into (at least) three equivalent portions so that a food or feed business may have a counter-analysis carried out. Should a dispute arise between the official and the counter-analysis a retained portion of the sample may, in statutorily defined circumstances, be submitted to the Government Chemist for a definitive investigation, see Figure 1[9]. The Government Chemist Programme[10] at LGC[11] provides a statutorily-based route of technical appeal to prevent or resolve measurement disputes prior to costly processes in the criminal justice system; it is the "referee function"[12]. The Programme, which also encompasses advisory and research work, is funded by the National Measurement Office[13] (NMO), an executive agency of BIS, the Department of Business Innovation and Skills. NMO and the Government Chemist are thus independent of central regulatory departments such as the Food Standards Agency (FSA) or the Department for Environment, Food and Rural Affairs (Defra). The NMO oversees governance of the Government Chemist

Programme through an advisory group made up of industry, enforcement and academic stakeholders. The need for referee analysis often arises in novel or complex interfaces between science and the law, hence considerable synergy is provided by the assignment of LGC as the UK's designated National Measurement Institute for chemical and bio-analytical measurement giving access to fundamental measurement science research and advanced instrumental techniques[14]. Referee analysis is recognised at European level as supplementary expert opinion (SEO) pursuant to Article 11(5) of Regulation 882/2004 on official controls. The Article states:

> *"The competent authorities shall establish adequate procedures in order to guarantee the right of feed and food business operators whose products are subject to sampling and analysis to apply for a supplementary expert opinion, without prejudice to the obligation of competent authorities to take prompt action in case of emergency."*

SEO could be obtained by the trader on the second portion of the sample at any laboratory other than the one that first analysed the sample. However on some occasions a food business may wish to approach the Government Chemist for SEO. In these circumstances, if the Government Chemist decides to accept the SEO request, the case must be handled with the full rigour of a referee case and only the retained (third) portion is accepted for referee analysis.

Figure 1 – Arrangements for Referee Analysis



This paper describes Government Chemist referee casework in the calendar years 2010 and 2011 and provides an opportunity to assess the performance of the technical appeal safeguard and the control system in the limited number of complex cases where appeal has been invoked.

Referee Cases Completed in 2010-11

Referee casework arises most frequently under the Food Safety Act 1990[15] or the Agriculture Act 1970[16]. When a referral is received a case meeting is convened to consider the problem, allocate resources and set a deadline for completion. Decisions on the appropriate analytical strategy are informed by consideration of the approaches, if known, taken by the enforcement and owner's laboratories but primarily by regularly updated awareness of the scientific and legal context in which the case has arisen. The default analytical strategy is multi-replicate analyses of the referee sample(s) on each of three days with analysis of reference material(s) (RM's), preferably certified (CRM's), blanks and spiked blanks to provide a high level of analytical confidence and a case-specific measurement uncertainty. All significant analytical steps are witnessed by a second scientist. If CRM's are unavailable, RM's or Proficiency Test samples are used. The resulting dataset is independently statistically evaluated, a certificate is drafted and/or reviewed by a qualified person (the Referee Analyst, MChemA[17]) and finally the case file is brought to the Government Chemist (or deputy) for peer review. If all steps are satisfactory the Government Chemist will allow the certificate to be released. It is important to recognise that, quite properly for an appeal function, the resource expended far exceeds that available to a Public Analyst or trade laboratory for routine analysis.

During 2010, 9 cases were referred to the Government Chemist – 6 in connection with the Food Safety Act, and the remaining 3 in accordance with the provisions of the Agriculture Act. In 2011, 16 cases were referred, 11 and 5 in connection with the Food Safety Act and the Agriculture Act respectively, see Tables 1 and 2.

Table 1 – Referee Cases 2010

Type of Product	Type of Enforcement Authority	Product	Issue
Food	Inland Authority	Beer	Quality
Food	Inland Authority	Turmeric oleoresin	Dimethyl yellow
Food	Inland Authority	Apples	Morpholine
Food	Port Health Authority	Almonds	Aflatoxin
Food	Port Health Authority	Prawns	Nitrofuran
Food	Port Health Authority	Prawns	Nitrofuran
Animal Feed	Inland Authority	Sheep feed	Se, Zn, Cu
Animal Feed	Inland Authority	Cattle feed Layer feed Pig feed	Fibre Mg Vitamin A
Animal Feed	Inland Authority	Wild bird feed (peanuts)	Aflatoxins

Table 2 – Referee Cases 2011

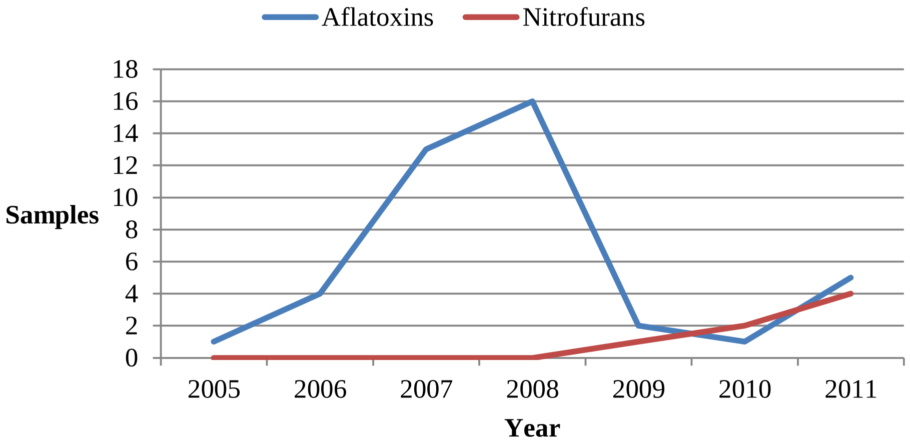
Type of Product	Type of Enforcement Authority	Product	Issue
Food	Inland Authority	Supplements	Opinion on format of certificate
Food	Inland Authority	Noodles	Aluminium

Type of Product	**Type of Enforcement Authority**	**Product**	**Issue**
Food	Inland Authority	Dry cure bacon	Nitrite and nitrate
Food	Inland Authority	Noodles	Aluminium
Food	Port Health Authority	Peanuts in shell	Aflatoxin
Food	Port Health Authority	Figs	Aflatoxin
Food	Port Health Authority	Prawns	Nitrofurans
Food	Port Health Authority	Rice	Aflatoxin
Food	Port Health Authority	Prawns	Nitrofurans
Food	Port Health Authority	Prawns	Nitrofurans
Food	Port Health Authority	Soft shell Crabs	Nitrofurans
Animal Feed	Inland Authority	Mineral feed	Cu, Mg, Co, Ca, Vitamins A & E
Animal Feed	Inland Authority	Wild bird feed (peanuts)	Aflatoxin
Animal Feed	Inland Authority	Cattle feed	Oil, Vitamins A & E
Animal Feed	Inland Authority	Horse feed	Oil, Vitamins A & E
Animal Feed	Port Health Authority	Wild bird feed (peanuts)	Aflatoxin

Mycotoxins – Aflatoxins

Aflatoxins remain prominent in casework although referrals have diminished from their high point in 2008 (Figure 2).

Figure 2 - Volume of Activity for Aflatoxins and Nitrofurans by Year



Note: some cases include more than one sample

Aflatoxins are mycotoxins, secondary metabolites produced by some fungal species, that can colonise crops such as peanuts, spices or figs and contaminate them in the field or after harvest[18]. Aflatoxins are genotoxic carcinogens[19,20] capable of inducing liver cancer particularly with simultaneous hepatitis B virus infection and are among the most potent mutagens known[21]. Stringent control measures are in place to reduce human consumption[22,23]. Since fungal contamination is patchy aflatoxins are known to be in-homogenously distributed in consignments necessitating incremental sampling at import, followed by high shear mixing with a defined amount of water to form a slurry before division into multi-part portions. If one of the multi-part portions fails to meet statutory limits the consignment is rejected[24,25].

Referee samples are analysed by extraction with acetonitrile/water or methanol/water depending on the matrix, immunoaffinity column clean-up and liquid chromatography, duplicate injection, with post column derivitisation (bromination or Kobra cell[26]) and fluorescence detection[27]. Sample extracts, together with solvent standards, pre-extraction and post-extraction matrix spikes, are also analysed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) to confirm the molecular identity and presence of aflatoxin B_1[28]. A set of moisture determinations is carried out to confirm, as far as possible, that the water/product mix was as stated by the Public Analyst.

A data set of 18 observations is created for aflatoxin B_1, and total aflatoxins (the sum of aflatoxins B_1, B_2, G_1, and G_2) from the means of duplicate injections, after correction for water added, recovery and, if required, the kernel to whole nut ratio. The data set is examined for outliers and homogeneity of variance, performing appropriate statistical tests where necessary. An analysis of variance is then performed on the data to obtain a standard error of the mean. The expanded measurement uncertainty, MU, is calculated as a 95% confidence interval on a case-specific basis derived from the standard error and a coverage factor based on the degrees of freedom. Rounding, which is left until the final step, is outwards hence, for example a mean and MU of 3.514 ± 0.452 $\mu g\ kg^{-1}$ yielding a 95 % confidence interval of 3.062 – 3.966 $\mu g\ kg^{-1}$ is reported as not less than 3.0 $\mu g\ kg^{-1}$ and not more than 4.0 $\mu g\ kg^{-1}$ [29].

Table 3 shows the summary results of the four aflatoxin in food cases dealt with in 2010/11 one of which was submitted in December 2009. In three of the cases no issue was taken with total aflatoxins for which there are, of course, also limits hence these are not reported here. In the figs case the Public Analyst found the concentration of total aflatoxins was slightly in excess of the limit of 4.0 $\mu g\ kg^{-1}$ and this was confirmed.

It is interesting to note that in the first two cases the analysis carried out on behalf of the consignment owner (not the same laboratory in the different cases) yielded means for aflatoxin B_1 above the limiting concentration of 2.0 $\mu g\ kg^{-1}$ aflatoxin B_1 however the means exceeded the limit by less than their associated MU thus providing a defence against a non-compliant finding. Figure 3 illustrates the findings in the first case (almonds) diagrammatically. As noted in Table 3 in three out of four cases the Public Analyst’s findings were upheld. In the last case the Food Business Owner’s findings that the sample contained negligible aflatoxins were confirmed.

Table 3 – Aflatoxin B_1 in Imported Food Cases 2010/11

Matrix	**No of sub-samples**	**Relevant sub-sample results, µg kg^{-1} aflatoxin B1 expressed on kernel where in-shell**			**Limit µg kg^{-1}**	**Outcome**
		Public Analyst	**Owner**	**Government Chemist**		
Almonds	3 (note 1)	3.0 ± 0.4	2.8 ± 0.8	3.5 ± 0.45	2.0	Public analyst's findings confirmed
Peanuts in shell	2 (note 2)	3.1 ± 0.8	2.3 ± 0.3	2.7 ± 0.5	2.0	Public analyst's findings confirmed
Figs	2 (note 3)	2.5 ± 0.3	(note 4)	2.3 ± 0.1	2.0	Public analyst's findings confirmed
Rice	1	2.9 ± 0.4	0.27 (note 5)	0.3 ± 0.09	2.0	Owner's findings confirmed

Note 1: Two sub-samples returned negligible results in both public analyst's and owner's laboratories and this was confirmed by the Government Chemist

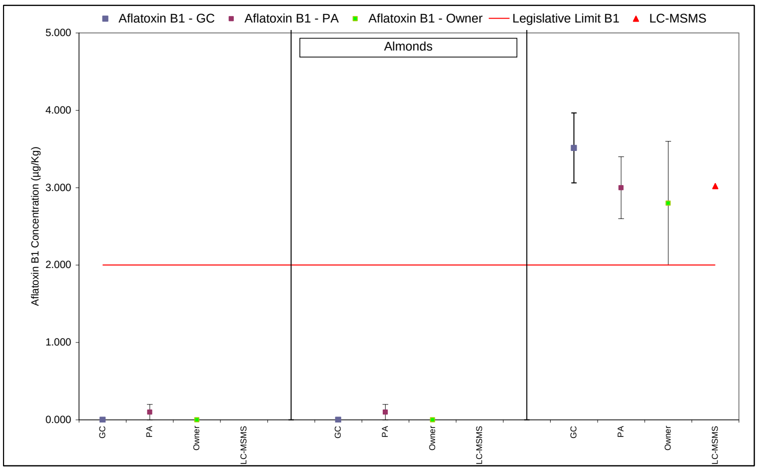
Note 2: One sub-sample returned negligible results in both public analyst's and owner's laboratories and this was confirmed by the Government Chemist

Note 3: One sub-sample returned negligible results in the public analyst's laboratory and this was confirmed by the Government Chemist

Note 4: The owner's laboratory was mistakenly instructed to homogenise both subsamples and returned a result for the composite sample of 0.7 µg kg^{-1} aflatoxin B_1

Note 5: No MU was reported

Figure 3 – Diagrammatic Representation of Aflatoxin B_1 Results in an Almonds Case



Note: the owner's result from the third sub-sample provides a defence

Table 4 shows the summary results of the three aflatoxin in feed cases dealt with in 2010/11; all concerned birdfeed.

Table 4 – Aflatoxin B_1 in Animal Feed Cases 2010/11

Matrix	**No. of samples**	**Relevant sample results, μg kg^{-1} aflatoxin B_1**			**Max Limit**	**Outcome**
		Agricultural Analyst	**Owner**	**Government Chemist**		
Peanuts	4 (note 1)	99.2 ± 12	1.17 (note 5)	239 ± 14	20 μg kg^{-1} = 0.02 mg kg^{-1} (note 4)	Agricultural analyst's findings confirmed in one sample
		33.0 ± 4	0.07 (note 5)	9.8 ± 2.5		
Peanuts	3 (note 2)	70 ± 20	6.0 (note 5)	14.7 ± 2		Owner's findings confirmed
Peanuts	4 (note 3)	12.8 ± 1.6	Not known	1.5 ± 0.9		Agricultural analyst's consignment findings confirmed but variation in each sample
		207 ± 25	3.40 (note 5)	23.9 ± 7.9		
		8.3 ± 1.0	Not known	66.1 ± 18.3		
		2.0 ± 0.2	Not known	75.9 ± 35		

Note 1: Two samples returned negligible results in both agricultural analyst's and owner's laboratories and this was confirmed by the Government Chemist

Note 2: Two samples returned negligible results in both agricultural analyst's and owner's laboratories and this was confirmed by the Government Chemist

Note 3: All 4 sample results are cited here

Note 4: The maximum limit for aflatoxin B_1 in feed is expressed in statute (see below) as mg kg^{-1} however for ease of reference the results cited in Table 4 are expressed as μg kg^{-1} rather than mg kg^{-1}

Note 5: No MU was reported

The statutory provisions in relation to the sampling of feed differ from those for food in that although incremental samples are taken and mixed there is no provision for high shear mixing with water to form a slurry. Thus it would be expected that the portions allocated to the Agricultural Analyst, the Owner and the Government Chemist might not exhibit the same degree of uniformity as those in food work. In view of the proven efficacy in homogenisation that slurrying with water brings about, each sample received for referee analysis was separately slurried prior to the analysis. We are not aware what, if any, slurrying homogenisation was undertaken by the Agricultural Analyst or laboratories acting for owners. The analysis and data handling were carried out as described above with the addition of moisture determinations before slurrying in order to report the results on a 12 % moisture basis.

The concentration of aflatoxins in animal feed, including that for oral feeding to animals living freely in the wild such as peanuts for wild bird feed, is regulated by provisions specific to each of the countries of the UK[30] implementing Regulation (EC) No. 767/2009[31] on the placing on the market and use of feed and Directive 2002/32/EC[32] on undesirable substances in animal feed. These measures provide for a maximum limit for aflatoxin B_1 of 0.02 mg kg^{-1}, relative to a moisture content of 12 %; there is no limit for total aflatoxins. Further, although sampling provisions extend to multiple samples from consignments depending on their size the regulations are silent on the interpretation in the event that some samples from the same consignment are compliant while others are not. In order to address this we took the view that samples that exceeded the limit of 0.02 mg kg^{-1} were unsafe within the meaning of Article 15 of Regulation (EC) No 178/2002. Article 15 (3) of Regulation 178/2002 stipulates that where a feed which has been identified as not satisfying the feed safety requirement is part of a batch, lot or consignment of feed of the same class or description, it shall be presumed that all of the feed in that batch, lot or consignment is so affected, unless following a detailed assessment there is no evidence that the rest of the batch, lot or consignment fails to satisfy the feed safety requirement. We added the rider that we considered that a detailed assessment of the rest of the consignment would in all probability demonstrate that it would fail to satisfy the feed safety requirement. None of our certificates were subsequently challenged on this aspect[33].

Although the above data are limited in their extent it is clear that there is much more dispersion in the aflatoxin results between laboratories in the peanut animal feed samples than in the food samples. Since largely the same laboratories are involved this suggests that sampling, and in particular the lack of a requirement for high shear mixing with water to form a slurry prior to splitting the samples into parts, is the main source of the variation.

Beer (Ale) – Unfit

Following a routine inspection by Local Authority Trading Standards officers in 2010 in a small retail outlet, a batch of bottled ale was discovered that was many years beyond its best before date (BBE Jan 2000). A formal sample was taken, the Public Analyst reported that the sample was unsatisfactory for taste, appearance and microscopy and a prosecution was mounted. The part of the sample left with the retailer was lost and since we were contacted about the matter close to the court hearing date we suggested an application to the Court. The Court duly remitted the matter to the Government Chemist and adjourned pending our report.

Two bottles of ale were received, opened aseptically and representative contents transferred to sterile, chemically-clean containers. Figure 4 shows the ale compared with control samples after transfer to sample vials in our laboratory.

Analysis was carried out as follows:

- appearance was judged visually, alcohol by volume (ABV) was determined after filtration by distillation and determination of the relative density of the distillate (Anton Paar density meter DMA5000) and reference to Laboratory Alcohol tables[34]
- original gravity (OG) was determined by methods established in the Laboratory of the Government Chemist based on relative density of the distillate and the residue after distillation made up to its original volume taking into account the acidity, if necessary[35,36,37,38,39]

- acidity was determined by titration against 0.1M sodium hydroxide to a phenolphthalein end-point
- transmitted light compound microscopy was carried out at magnifications between 40x and 400x in aqueous mounts of the sample and of the suspended matter isolated by centrifugation
- microbiological and other examination was carried out under the supervision of the authors at Campden BRI, formally Brewing Research International[40]
- haze was measured after the samples had been degassed by placing on a side-to-side shaker at 100 cycles per minute for 35 minutes by using a Dr Lange LTP6B 90° double-beam stray light beer photometer measuring at 860 nm and calibrated in EBC (European Brewing Convention) formazin units
- colour was measured after filtering the samples through a 0.45 µm syringe filter in a spectrophotometer at 430 nm in 10mm disposable cells
- total viable counts were determined by plating 5 mL of sample in duplicate respectively onto WLN agar, incubating for five days at 25°C and Raka Ray agar, incubating for five days at 25°C under anaerobic conditions and counting the colony forming units (CFU) produced.

Figure 4 – "Unfit" Ale after transfer to vials

Note: the two outer vials contain the referee sample while the three inner vials contain controls

Table 5 summarises the findings.

Table 5 – Findings in Respect of Bottled Beer

	Public Analyst	Government Chemist: Sample A	Government Chemist: Sample B
Appearance as received	The beer was brown, very cloudy and the results of a microscopical inspection indicated the presence of some bacteria indicating that the beer had undergone some deterioration	An unopened brown glass bottle with crown cap closure; examined visually and by light transmitted through the bottle the contents were seen to be markedly turbid, with suspended matter freely distributed throughout the bulk of the ale and tending to partially settle on standing.	

	Public Analyst	**Government Chemist: Sample A**	**Government Chemist: Sample B**
Appearance on and after opening	Taste - Unsatisfactory	On opening, the contents appeared to be normally pressurised and on decanting were markedly turbid, the samples were not tasted.	
Alcohol by volume	4.4 %	4.44 %	4.40 %
Original gravity	not available	1040.3	1040.1
Acidity as acetic acid	0.13 g 100mL^{-1}	0.14 g 100mL^{-1}	0.18 g 100mL^{-1}
Microscopy of suspended matter	"presence of some bacteria"	Abundant amorphous fine sub-micron particles and agglomerates of same	
Haze, EBC Formazin Units	not available	Off scale; >20 EBC Units	Off scale; >20 EBC Units
Colour	not available	54 EBC Units	53 EBC Units
Total Viable Organisms in aerobic conditions (Note 1)	not available	nil <1 CFU per 5 mL	nil <1 CFU per 5 mL
Total Viable Organisms in anaerobic conditions (Note 2)	not available	nil <1 CFU per 5 mL	nil <1 CFU per 5 mL

Note 1: per 5 mL at 25°C in 5 days on WLN agar
Note 2: per 5 mL at 25°C in 5 days on Raka Ray agar
CFU – Colony Forming Units

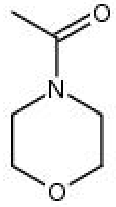
The measurement of haze, a turbidity resulting from interactions between proteins and phenolic compounds in beer and ale, is a standard means of assessing quality. Literature data[41] confirms that haze values of less than 2 (as measured herein) are considered normal in bright beers and ales of acceptable quality. Haze may be a multifactorial problem in beer and ale but is known to develop on storage. There was no other evidence of deterioration; no viable microorganisms were recovered from the sample, the alcoholic strength conformed to the declared value and the acidity was normal. We concluded that owing to the presence of excessive haze consistent with deterioration of the ale, the sample was unfit for human consumption within the meaning of Article 14(2)(b) and Article 14(5) of Regulation (EC) No

178/2002 on the general principles and requirements of food law. When the matter was heard the defendant decided on the day of the trial to plead guilty and was fined £4500 plus £3187 costs.

Morpholine in Apples

In autumn 2010 the UK Food Standards Agency revealed that the additive morpholine had been found on some apples imported from Chile into the UK. Food businesses were advised that apples treated with wax containing morpholine should not enter the UK food supply. It subsequently came to light that morpholine may also have been used on citrus fruits but since these are peeled before consumption the FSA did not require citrus fruits to be taken off the shelves or ask consumers not to eat them. Morpholine is a cyclic secondary amine ether (Figure 5).

Figure 5: Morpholine



It is a precursor of carcinogenic nitrosamines and although the possibility of their formation in the human stomach after ingestion of treated apples is reported as highly unlikely[42] it has not been authorised as a food additive in the EU. Methods for its detection are required since it is permitted in other jurisdictions and may be present on food through direct treatment of fruit with waxes containing the compound, through steam treatment during processing or from packaging. Methods using derivatising agents with the inclusion of UV chromophores such as dansyl chloride yield good separation and high sensitivity but with mass spectrometric fragment ions predominantly originated from the derivatising group rather than the morpholine moiety hence forensic conformation of the presence of morpholine was lacking. Against that background the Government Chemist was asked for a second opinion on a consignment of imported apples in which a Public Analyst had found morpholine. Although the consignment was re-exported we recognised that with no official method there is a risk of non-specific positives and problems may ensue unless a forensically robust confirmatory method is made available to Public Analysts. Accordingly we developed an amine acetylation derivatisation method from which fragment ions originating from the morpholine group can be detected using GC-MS or LC-MS.

Table 6 shows the good agreement between the GC-MS and LC-MS results and the Public Analyst’s LC-UV findings. The method, which requires further validation is published elsewhere[43].

Table 6 – Morpholine Findings in Apples

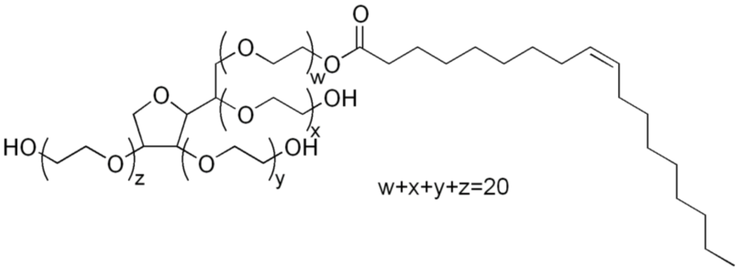
Public Analyst	Government Chemist
2.0 mg kg^{-1}	GC-MS: 1.48; 1.18 mg kg^{-1} LC-MS: 1.72; 1.23 mg kg^{-1} Overall mean: 1.4 mg kg^{-1}

Illegal Dyes – Dimethyl Yellow

The presence of illegal dyes such as the Sudan reds in spices is a well-recognised problem and numerous methods of analysis have been described for their determination. However some spice derived matrices present particular problems. Oleoresins, complex extracts of spices containing phenolic natural pigments and terpene-like lipids, are used in the formulation of oriental sauces, pickles and processed spiced meals and hence are likely to penetrate far into the food chain[44]. In 2010 a Public Analyst reported suspicions of the presence of the illegal dye dimethyl yellow in a turmeric oleoresin. Although this turned out to be a false positive finding quickly resolved by the Public Analyst using LC-MS/MS it had the potential to trigger a costly food recall. The LC-MS/MS investigation came at the cost of subsequent decontamination of the instrument, incurring significant down time. The Government Chemist was asked to look at the problem.

It was agreed by the European Commission in 2009 that LC-UV methods should be used for the routine quantification of illegal dyes in food. However our findings in this case show that LC-UV *simpliciter* for the analysis of illegal dyes in spice oleoresins cannot deal with the interferences present. In particular the presence of a surfactant (e.g. Polysorbate 80, Figure 6) in oleoresins to aid dispersion in foods causes analytical problems even for advanced LC-MS/MS systems. We developed a combination of liquid/liquid extraction, gel permeation chromatography and silica solid phase extraction with LC-MS/MS that yielded a forensically robust determination of dimethyl yellow in an oleoresin/surfactant mixture. A limit of detection below the current *de facto* European action limit of 500 µg kg^{-1} proved possible. Validation work would be required to put the method into routine use and extend it to other illegal dyes. This would also afford an opportunity to explore further the use of disposable GPC columns as a cost effective option. Further details are published elsewhere[45].

Figure 6 – Polysorbate 80



Nitrofurans

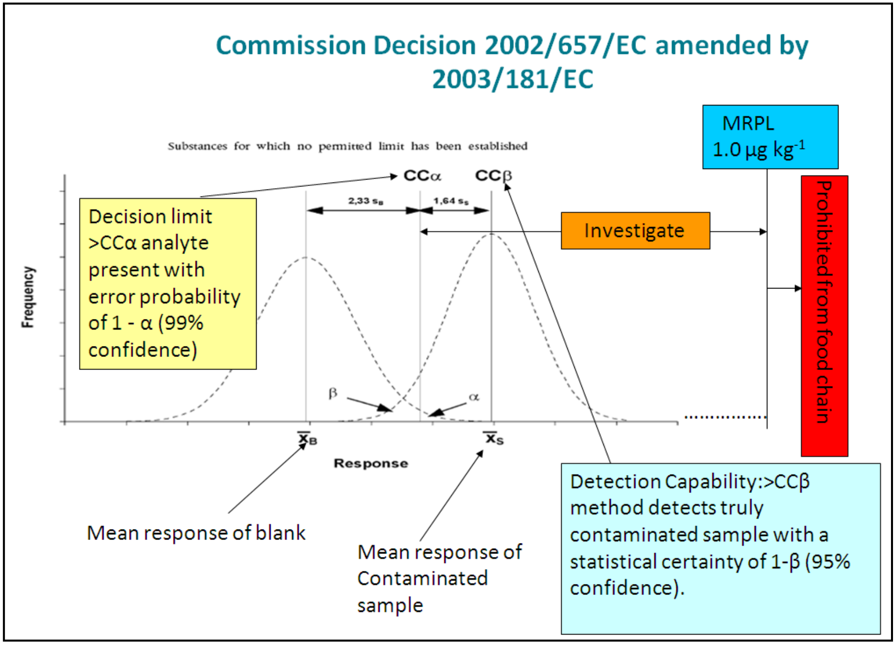
Once widely used as veterinary antibiotics, these compounds are now prohibited in food in the EU owing to concerns about the carcinogenicity of their residues in edible tissue[46,47] Nitrofurans are unstable in animal tissue, but give rise to carcinogenic metabolites, which are measured as marker compounds, shown in Table 7[48].

Table 7 – Nitrofuran Drugs and their Marker Metabolites

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

Law that requires the absence of an illegal substance in food raises a problem for analytical chemists when reporting results but a pragmatic solution has been adopted by the European Commission. Food of animal origin containing a nitrofuran metabolite fails to comply with Community legislation and is prohibited from entering the food supply chain unless the concentration of such a metabolite does not equal or exceed a reference point for action pursuant to Regulation 470/2009[49]. Such a reference point for action for nitrofuran metabolites has been established[50] as a minimum required performance limit, MRPL, of 1.0µg kg^{-1}. Where the results of analysis confirm the presence of a nitrofuran metabolite below the reference point for action but above a decision limit, CCα determined as part of the analytical procedure[51], an investigation is required on the part of the competent authority, see figure 7.

Figure 7 – Regulation of Nitrofurans – (substances for which no permitted limit has been established)


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

Decision limit >CCα analyte present with error probability of 1 - α (99% confidence)

Detection Capability:>CCβ method detects truly contaminated sample with a statistical certainty of 1-β (95% confidence).

Moreover, whereas aflatoxin measurement is a mature science with widespread analytical capability, nitrofuran marker compound analysis presents many pitfalls and is confined to relatively few laboratories owing to the complex nature of the analysis. Metabolites are extracted by acid hydrolysis, converted using *ortho*-nitrobenzaldehyde to their nitrophenyl derivatives and determined against deuterated standards by liquid chromatography with tandem mass spectrometry, LC-MS/MS. There is a need to measure free and bound metabolites[52] and one in particular; the semicarbazide (SEM) presents problems of interpretation. SEM has been found in breadcrumbs and other bread products arising from the presence of azodicarbonamide, a flour treatment agent also banned in the EU but still used in Asia[53]. Azodicarbonamide is also known to be used as a blowing agent for plastic gaskets, and SEM is also thought to arise from carrageenan and through hypochlorite treatment of nitrogen-containing foods[54].

Research published in 2011 demonstrated that SEM is naturally occurring in crustacean shells[55,56] A solution, difficult to put into practice routinely, is to excise and test the inner core flesh of the animal thus avoiding shell-derived SEM migrated into the outer flesh.

Lastly, procedures for sampling food of animal origin to test for antibiotic residues such as nitrofurans differ from those for contaminants such as mycotoxins in food of non-animal origin. For practical reasons and to guard against loss of analyte the homogenisation of food of animal origin cannot be as comprehensive prior to division of the sample into three portions. Thus any inhomogeneity in the distribution of antibiotic residues may be reflected in the results obtained by each laboratory carrying out an analysis.

Having initially seen cases in 2009, in 2010 we investigated two cases of disputed findings of nitrofuran marker residues in imported food followed by four cases in 2011. Details are shown in Table 8; in five out of the six cases in 2010/11 we overturned the OCL findings. In three of the cases we reported a nitrofuran marker metabolite to be present but at a concentration less than the reference limit at which the consignment would be banned from entering the food chain. Inspection of Table 8 shows that our thinking developed during 2011 as a result of information published that year that SEM is naturally present in the shells of crustaceans and in the latter two cases in the series we based our conclusions on SEM on the analysis of core flesh only.

As a consequence of the developing insight a comprehensive advice note on nitrofuran analysis was published[57] since when the number of disputes has diminished with only one in 2012 in which the OCL findings were upheld.

Table 8 – Nitrofuran cases 2010/2011

Matrix and case information	Nitrofuran metabolites µg kg^{-1} see table 7 for key to abbreviations								Summary
	Public Analyst				Government Chemist				
	AOZ	AMOZ	AHD	SEM	AOZ	AMOZ	AHD	SEM	
Tiger Prawns SEO	2	<0.5	<1	<1	0.42 ±0.06	<0.3*	<1.0*	(Note 1)	AOZ found but <MRPL
Tiger Prawns SEO	4	<0.5	<1	<2	0.92 ±0.78	<0.5*	<1.0*	1.60±0.22	SEM found above MRPL
Tiger Prawns SEO	1.3	<0.5	<1	<1	0.68 ±0.15	<0.3*	<0.9*	(Note 1)	AOZ found but <MRPL
Tiger Prawns SEO	2.5 ±0.8	<0.5	<1	<1	0.99 ±0.35	<0.4*	<1.0*	(Note 1)	AOZ found but <MRPL
Tiger Prawns Dispute	<0.5	<0.5	<1	2.8 ±0.8	(Note 2)			Outer flesh 0.49±0.09	SEM present
Owners results	<0.5	<0.5	<0.5	<0.5				Core flesh <0.54*	SEM not detected
Soft Shell Crab SEO	<0.5	<1	<1	2.2±0.7	Note 2			Core flesh <0.58*)	SEM not detected

*CCβ

Note 1: Nitrofuran metabolites were determined as their total (bound plus unbound) derivatives. This approach is suitable for measuring AOZ, AMOZ and AHD. It is unsuitable for measuring SEM in prawns owing to the possibility of unbound SEM occurring naturally in high concentrations in the shell, and in lower concentrations in the tissue close to the shell. SEM was not in dispute in this case and since good analytical practice is that SEM should only be quantified using methods that distinguish the bound residue and therefore SEM was not reported.

Note 2: The results AOZ, AMOZ and AHD were examined and by inspection there was no evidence of the presence of these metabolites hence their datasets were not processed further in order to expedite the resolution of the case.

Aluminium

Spanning three centuries of food safety regulation the question of the safe amount of aluminium in food was raised again as a new topic for referral in 2011 illustrating that nothing ever really goes away in food chemistry. Two cases arose in 2011 regarding imported noodles.

The use of alum (hydrated potassium aluminium sulphate) as an adulterant to whiten wheat flour and bread was a *cause célèbre* in the 19th century. Aluminium in food was the focus of a great deal of attention for a time in the late 20th century when a link between aluminium intake and the development of Alzheimer's disease was postulated. Parenteral exposure to high aluminium concentrations by patients undergoing dialysis has led to neuro-toxicity however the link with Alzheimer's disease is no longer considered as relevant[58,59]. However, in November 2008 elevated concentrations of aluminium in imported noodles were discovered in Germany[60]. These and other findings suggested the illicit use of aluminium based additives. As a consequence, EU law to require enhanced surveillance of noodle imports was enacted.

Importers in the UK voiced concerns that there may be laboratory bias in the measurement of aluminium in noodles and that wheat grown in China may naturally contain higher concentrations of aluminium than wheat grown in Europe and North America. Hence the two cases in 2011, one SEO and the other disputed findings.

The samples consisted, on our receipt, of powders which were further homogenised by grinding with a mortar and pestle. Three replicates of the well mixed sample were analysed for aluminium on each of three days by microwave pressure digestion with nitric acid and hydrogen peroxide followed by inductively coupled plasma mass spectrometry (ICP-MS) of the aluminium isotope ^{27}Al alongside reagent blanks, calibration standards, spiked samples and the Standard Reference Material, NIST 1547 "peach leaves". Moisture was also determined by loss on drying at 103±2°C. In the second case our recovery was 108% and the result was corrected for this. As far as we are aware the Public Analyst employed a similar technical approach with presumably fewer replicates. The laboratory acting for the owner in the second case also employed microwave pressure digestion but may have included hydrofluoric acid in the digestion mix. In each case our findings upheld those of the Public Analyst. Table 9 shows our findings.

Table 9 – Aluminium in Noodles, 2 Cases

Public Analyst		**Owner**		**Government Chemist**	
Result Al mg kg^{-1}	**Method**	**Result Al mg kg^{-1}**	**Method**	**Result Al mg kg^{-1}**	**Method**
13	ICP-MS	7.3* (Noodles) 8.9* (wheat flour)	ICP-OES	12.9 ± 0.27 (moisture 3.7 %)	ICP-MS
13.0 ± 2.3	ICP-MS	9.62	ICP-MS	11.9 ± 1.1 (moisture 7.8 %)	ICP-MS

*Pre-export certificates. Where no MU is stated none was reported

There is no domestic or European statute setting out a maximum concentration for aluminium in noodles and in this situation in the past the Government Chemist might have confined himself to stating an analytical result. However, recognising that it would be more helpful to propose expert interpretation, the evidence was assessed including LGC findings on aluminium in food. The European Food Safety Authority (EFSA) reported that in animal studies aluminium has the potential to produce neurotoxicity, embryotoxicity and reproductive toxicity[61]. EFSA and the Joint Expert Committee on Food Additives (JECFA) have also stated that aluminium intake may exceed recommended tolerable weekly intakes. The view taken by the European Commission supported by the Standing Committee on the Food Chain and Animal Health was[62]:

> *"Following requests for clarification as regards the proposed listing of noodles from China under Annex I to Regulation (EC) No 669/2009, the Commission clarified that, based upon available data, the level of 10 mg/kg could be used to distinguish noodles with acceptable unavoidable background presence of aluminium from noodles presenting unacceptable levels. Vote: qualified majority by 338 votes in favour, 7 votes abstained."*

Thus the Government Chemist certificates of analysis carefully stated the evidence in support of a limit for aluminium in noodles of 10 mg kg^{-1}. This was exceeded in both cases, and the consignments were refused entry into the UK.

However, in order to explore the issues and prompt further debate an advice note on aluminium in noodles was published[63].

Nitrite

It was noticed centuries ago that in curing meat certain batches of salt produced a pink colour and a special flavour, changes originally thought to arise from reaction with nitrate (saltpetre), present in some salt. Nitrite, added and formed from nitrate by bacterial action, is the true precursor of the flavour and colour changes, as well as contributing a preservative function. Caution around the toxicity of excess nitrate and nitrite has led to limits on the concentration of these compounds in cured meat, and analytical surveillance of food for nitrate and nitrite has a long history. The Government Chemist was asked for sampling advice to produce three equivalent portions of the official sample in the special case where hand-cured ham was being produced with concomitant uneven distribution of nitrite throughout the meat. Having followed the sampling advice, there still remained significant differences in the analytical results obtained by the OCL and trade laboratories and referee case analyses tended to support the OCL results. The concentration of nitrate and nitrite in cured meat is known to vary with time with the depletion of nitrite being more marked than nitrate[64]. Honikel[65] and Sebranek and Bacus[66] have described the chemistry of nitrogen compounds especially in relation to cured meats and the influence of other additives such as ascorbate on the fate of nitrite added to food. However the literature seems sparse on time-dependent changes in nitrite and nitrate in cured meat products although it is well known that this takes place.

Animal Feed

There have been many examples of food safety incidents originating from animal feed, hence regulation of feed continues to be an important facet of enforcement activity and referee casework inevitably arises. Cases involving aflatoxins in wild birdfeed have been dealt with above. As regards farmed animal feed there were two cases (4 samples) in 2010 and three (4 samples) in 2011. The analytes were metals (Co, Cu, Se and Zn), minerals (Ca, Mg), proximate (fibre and oil) and vitamins A and E. Vitamin analysis frequently combines wet chemistry with the instrumental techniques. It is both labour-intensive and technically demanding and, perhaps unsurprisingly, controversy remains between trade and enforcement interests on the techniques and their results. Referee analyses upheld OCL findings but also prompted further work aimed to resolve some of the issues arising and provide more facile and efficient approaches to vitamin analyses. A further paper on animal feed casework is in preparation.

Professional Standards

Lastly, in this review, in 2011 at the request of a trader, a desk top review was conducted of the format and content of Public Analyst’s certificates on lead in food supplements. Appraised against professional standards the certificates were found fit for purpose but improvements were suggested.

Conclusions

Referee casework, the demand led core of the Government Chemist’s function, safeguards consumers, enforcement laboratories, regulators, the agrifood sector and the courts from unwitting errors in measurement science. The even-handed credibility of the referee role is maintained by stringent governance of the function, painstaking analytical rigour and regularly updated awareness of the contextual science and legislation. The cases surveyed in this paper demonstrate that analytical results must be interpreted in increasingly complex scientific legal and policy contexts. The diversity of measurement methods surveyed in the paper is evident and indeed represents only a fraction of the range of techniques OCLs and the Government Chemist must competently deploy to ensure the system is effective and responsive.

Cases stemmed from both inland local authorities and port health authorities with the ratio tending more to the latter in 2011. The breakdown is presented in Table 10.

Table 10 – Origin of Referee Casework

Inland Authority	Port Authority
2010	
6 (66.7%)	3 (33.3%)
2011	
8 (50%)	8 (50%)

Analytical results tend to be disputed in the more complex cases, where results are close to legal limits or where there are recognised scientific uncertainties hence the trends herein cannot be extended to the chemical OCL system as a whole. However in general we tended to confirm Public Analysts' and Agricultural Analysts' findings. In 2010 the findings of the OCL system (Public Analysts and Agricultural Analysts) were upheld in 8 (88.9 %) out of 9 cases. In 2011 the findings of the OCL system were fully upheld in 10 (62.5 %) out of 16 cases.

The OCL system in the UK faced continuing funding challenges in 2010 and 2011 but in general performed well in areas where capability has been developed such as in aflatoxin analysis. Public Analysts' and Agricultural Analysts' findings were confirmed on technical appeal in 5 out of 7 (71.4%) cases. However much more dispersion is evident in aflatoxin results between laboratories in animal feed samples than in the food samples. Since largely the same laboratories are involved it is clear that sampling, and in particular the lack of a requirement for high shear mixing with water to form a slurry prior to splitting the samples into parts, is the main source of the dispersion. It is recommended that sampling and sample preparation should be harmonised in the feed and food areas.

OCL performance was less good in the more problematic area of drug residue analysis. Of six nitrofuran marker metabolite cases (all on imported crustaceans) only one (17%) was completely upheld. In 3 cases (50%) a residue was confirmed present but at a concentration below the limit at which the consignment should be prohibited from entry into the UK. Research published in 2011 demonstrated that the marker for nitrofurazone, semicarbazide SEM, is naturally occurring in crustacean shells and in two cases (33%) the Government Chemist confirmed SEM not detected in the core flesh of the animals overturning the OCL findings. The Government Chemist published a comprehensive advice note on nitrofuran analysis and since then the number of disputes has diminished with only one in 2012 in which the OCL findings were upheld. It is recommended that proposed better markers for nitrofurazone, such as its cyano derivative[67], should be further investigated and that the sampling of imported crustaceans should be reviewed to achieve better homogenisation prior to sample subdivision.

In animal feed analysis issues persist around tolerances, vitamin determinations and interpretation of Regulation (EC) No. 767/2009 which will be the topics of a separate case study paper.

The referee function remains a service clearly required in the UK. Going beyond the particular resolution of each case, active engagement in knowledge transfer of the issues arising enhances the capability and preparedness of the UK official control system for feed and food and demonstrates an even handed approach to all stakeholders. Continuing dialogue with regulators and trade interests has demonstrated the usefulness of this approach.

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Any view, information or advice given should not be taken as an authoritative statement or interpretation of the law, as this is a matter for the courts.

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