Assessment of laboratory performance for determination of PAHs in food – an important quality measure

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Introduction

PAHs are a group of compounds some of which are known or suspected genotoxic carcinogens. As such there is no safe level of exposure and intake from food should be as low as reasonably practicable (ALARP). PAHs have been known for a long time to occur at low levels in food [[i]], especially where food is smoked or dried during the production process. Recently there have been a number of incidents such as the problem where PAHs were found in olive pomace oil within the EU, and the occurrence of PAHs in cocoa butter, which have highlighted this issue. Legislation has been introduced within the EU that establishes maximum limits for benzo-a-pyrene legally allowed in food, with an instruction to monitor other PAHs with a view to including these in future legislation.

Product	Maximum level (µg/ kg wet weight)
Oils and fats intended for direct human consumption or use an as ingredient in foods ([2])	**2**
Foods for infants and young children	**1**
Baby foods and processed cereal-based foods for infants and young children([3])	
Infant formulae and follow-on formulae, including infant milk and follow-on milk([4])	
Dietary foods for special medical purposes([5]) intended specifically for infants	
Smoked meats and smoked meat products	**5**
Muscle meat of smoked fish and smoked fishery products([6])	**5**
Muscle meat of fish([7]), other than smoked fish	**2**
Bivalve molluscs, crustaceans, cephalopods, other than smoked	**5**
Smoked bivalve molluscs	**10**

There is a continuing need for laboratories conducting such analyses to demonstrate performance and reliability in laboratory analyses. Proficiency testing is one of three essential elements ensuring laboratory quality assurance, which is important where scientific judgements on food safety and quality are under continuous scrutiny by consumers and the media. Together with laboratory accreditation demonstrating compliance with the requirements of BS EN ISO/IEC 17025 [[ii]] (previously the series of EN45000 standards [[iii]]) and the use of methods validated by collaborative trials, proficiency testing assists laboratories in demonstrating performance and reliability. The analysis of an external quality check sample as part of a laboratory's routine procedures provides objective standards for individual laboratories to perform against and permits them to compare their analytical results with those from other laboratories. Thus, the participation in proficiency testing schemes is crucial to the independent assessment of laboratory performance. Indeed the lack of an independent assessment of the quality of data being produced in consumer safety related areas would hamper the work of enforcement authorities, would limit the scope and reliability of surveillance work on the food supply and would prejudice the mutual recognition of results and certificates.

Between May 2001 and May 2004, four proficiency tests were completed in an ongoing programme of the UK Food Analysis Performance Assessment Scheme (FAPAS®) for the analysis of a range of polycyclic aromatic hydrocarbons (PAHs) in food matrices including fish paste and olive oil. Figure 1 describes the proficiency testing process. Laboratories from approximately 20 countries throughout the world requested homogeneous test material samples, with between 14 and 46 submitting results for each test. Results were analysed by appropriate statistical procedures and z-scores were awarded for reported values[iv]. The target standard deviations were derived from the Horwitz equation[v]. Participants were encouraged to use analytical procedures routinely used in their own laboratory and to provide details of these methods. Information submitted included details of sample preparation for both HPLC and GC methods, with a brief description of the column and detector used, whether methods were accredited, and details of any internal standardisation.

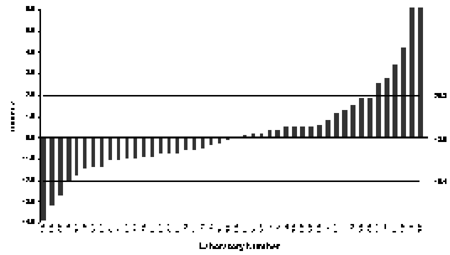

Figure 1: A flow diagram of the proficiency testing process

Figure 2: Histogram of data submitted for benzo(a)pyrene in proficiency test 4

Test Materials and Analytes

The suitability and quality of the test materials distributed are important to the effectiveness of a PT scheme. The two main criteria for a suitable test material are:

· It is important to choose test materials which resemble real samples as closely as possible. The ideal test materials are therefore real food samples naturally contaminated with the analyte in question.

· Successful PT and the comparison of participants' results can only take place if each participant receives an identical test material. A strict homogeneity testing procedure is used by which randomly selected units from a fully homogenised bulk test material are tested for each relevant analyte. Only when the resulting data pass the appropriate statistical tests which demonstrate sufficient homogeneity[vi] is the test material used for a proficiency test.

The test materials for the four FAPAS® proficiency tests for PAH analysis were endigiounously contaminated food samples: one fish paste and three olive oil samples.

Performance Assessment

The assessment of a laboratory's performance is given by an assigned z-score which is defined as:

z = (x-$\hat{x}$)/1

Where x is the measurement of analyte concentration in the test material,

$\hat{x}$ is the assigned value, the best estimate of the "true" concentration of the analyte

and 1 is the target value for standard deviation.

The assigned value, $\hat{x}$, is usually obtained from the robust mean[vii] of the entire data population for the analyte in question. Target values for standard deviation (s) are obtained from collaborative trial data (where available), from the use of the Horwitz equation [vii] or from the use of the modified Horwitz equation if the concentration of the analyte concentration in question is <120 mg/kg.[v]

Results

Reports for individual participants were prepared and laboratories assigned a z-score for each submitted result (see above). The reports show z-score markings in both tabular and ordered histogram format. Results from the four proficiency tests run by FAPAS® are given in Table 1. The histogram of results submitted for benzo(a)pyrene in test PT4 is shown in Figure 2.

In PT, each laboratory is free to choose a method of analysis. It is therefore possible to make correlations between methods used and performance. The identities of laboratories taking part in PT remain confidential at all times. Participation in a PT scheme is an indication of a commitment to quality. It is important therefore to understand the limitations of this external means of quality assessment when assessing the competence of a laboratory. If it is assumed that the assigned value ($\hat{X}$) and the target standard deviation (σ) correctly describe the variation of normally distributed participants' results (x), the data are known as "well behaved". Satisfactory performance in a proficiency test is indicated by a z-score in the range between –2 and +2. If a participant's z-score lies outside $|z| > 2$, there is a 1 in 20 chance that the result is in fact an acceptable result from the extreme of the distribution, and about a 1 in 300 chance that a z-score of $|z| > 3$ is actually acceptable. Close examination of the data submitted indicates that performance has improved significantly during the course of these tests and is now generally acceptable in terms of accuracy.

Table 1 shows that there is an increase in both number of participants and the proportion that achieve satisfactory z-scores over the four rounds. This could be an indication that performance is improved as a result of participation in proficiency testing, or may reflect the higher levels of PAHs found in the materials used for the later rounds.

Table 1: Details of test materials, assigned values and results from four FAPAS® proficiency tests

Proficiency test and date	Matrix	PAHs	Assigned value (µg/kg)	No of results submitted	% satisfactory
PT1: May 2001	Fish paste	Benzo(b)fluoranthene	3.07	12	58
		Benzo(a)pyrene	2.36	14	57
		Benzo(g,h,i)perylene	1.30	8	50
		Fluorene	65.3	9	56
		Fluoranthene	57.4	12	75
PT2: March 2003	Olive oil	Benzo(b)fluoranthene	*4.41**	20	*60**
		Benzo(a)pyrene	2.36	29	66
		Benzo(g,h,i)perylene	2.36	17	88
		Fluoranthene	25.8	16	81
		Chrysene	22.3	18	83
PT3: September 2003	Olive oil	Benzo(b)fluoranthene	17.6	31	65
		Benzo(a)pyrene	12.8	44	89
		Benzo(g,h,i)perylene	10.6	30	73
		Chrysene	*112**	28	*93**
		Benz(a)anthracene	34.7	29	62
PT4: May 2004	Olive oil	Benzo(b)fluoranthene	24.90	32	72
		Benzo(a)pyrene	18.66	46	80
		Benzo(g,h,i)perylene	17.83	31	77
		Benz(a)anthracene	38.75	32	91
		Indeno(1,2,3-cd)pyrene	11.08	27	81

* The uncertainty of the assigned value for these compounds was large, so assigned values and z-scores were given for information only.

[i]**References**

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[ii]iii European Standard EN 45000 Series CEN/CENELEC 1989

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