

SUMMARY

1. The test material for FAPAS[®] proficiency test 3023 was dispatched in September 2009. Each participant received a chocolate test material to be analysed for melamine. In total, 83 sets of test material were distributed to participants in 37 countries. Of these, 72 participants, i.e. 87%, returned results for the analyte within the time-scale demanded by the Scheme.
2. The assigned value (\hat{X}) for melamine was calculated from the most appropriate measure of central tendency of participants' results [1, 2].
3. The target standard deviation (σ_p), calculated using the appropriate form of the Horwitz equation [3], was used in conjunction with the assigned value (\hat{X}) to derive z-scores for participants' results. z-Scores are considered satisfactory if $|z| \leq 2$.
4. Results for this proficiency test are summarised as follows:

analyte	assigned value, \hat{X} , mg/kg	number of satisfactory z-scores, $ z \leq 2$	total number of z-scores	satisfactory, %
melamine	5.69	49	72	68

5. Surplus test materials from this test are not available for sale.
6. Whereas this Report has been produced in good faith and in accordance with best industry practice, neither the Food and Environment Research Agency nor the Secretary of State for Environment, Food and Rural Affairs accepts any liability whatsoever as to the application or use of the information contained therein.

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1. INTRODUCTION

1.1. Proficiency Testing

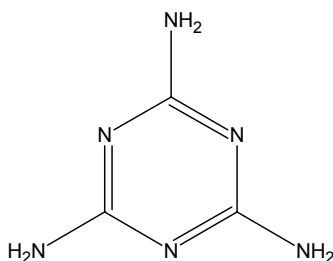
The demand for independent proof of competence from regulatory bodies and customers means that proficiency testing is relevant to all laboratories testing food and feed for quality and safety in every country. Hence, it is a requirement of accreditation to ISO 17025 [4] that the laboratory takes part in a proficiency testing scheme, if a suitable scheme exists. Further, for laboratories entrusted with the official control of food and feeds, Article 12 of EU Regulation (EC) 882/2004 [5] requires such laboratories to be assessed and accredited in accordance with ISO 17025, i.e. proficiency testing is a legal requirement for these laboratories. Thus, together with the use of validated methods, proficiency testing is an essential element of laboratory quality assurance.

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. Such standards and comparisons can go beyond the actual chemical analysis. For example, the ability to report results in specified units and within a given time scale are important aspects of quality. Hence, participants in FAPAS[®] who submit results after the closing date of a proficiency test are only included in the statistical evaluation if there are extenuating circumstances.

It is important to understand the statistical limitations of this external means of quality assessment when gauging the competence of a laboratory. The results of a typical chemical analysis will be normally distributed. That is to say, the majority of results will be centred on a mean value but, inevitably, some results will lie at the extremes of the distribution. The statistics of a normal distribution mean that about 95% of data points will lie between a z-score of -2 and +2. Performance in a FAPAS[®] proficiency test, therefore, is considered 'satisfactory' if a participant's z-score lies within this range. It follows that if a participant's z-score lies outside $|z| > 2$ there is about a 1 in 20 chance that their result is in fact an acceptable result from the extreme of the distribution. If a participant's z-score lies outside $|z| > 3$ the chance that their result is actually acceptable is only about 1 in 300.

Full details of the FAPAS[®] proficiency testing scheme is available via our Protocols [6, 7].

1.2. Melamine



Melamine

Melamine (2,4,6-triamino-1,3,5-triazine) is a synthetically produced chemical used for a wide variety of applications, including plastics, laminates, adhesives, paints, textile finishes, paper coatings and fertilizer mixtures. Melamine contains about 66% by mass of nitrogen and the

addition of melamine to foods can lead to a false increase in the apparent protein content when a non-specific procedure, such as the Kjeldahl reaction, is used to assess protein content by nitrogen measurement. There are no approved uses for the direct addition of melamine to food (e.g. in Europe and the United States of America) [8].

Melamine adulteration was reported in the literature when a spate of pet food recalls began in 2007 after a number of manufacturers discovered a contamination, which appeared to be causing illness and even death to pets consuming their products. In 2008 a health scare concerning the adulteration of milk and milk products (including infant milk) with melamine in China prompted investigations worldwide into the concentrations of melamine and, in some cases, related analogues (e.g. cyanuric acid) in milk, milk ingredients and composited products containing milk-derived ingredients.

Many countries have introduced limits for melamine in infant formula and foods. Recent EU rules [9], [10] imposed special conditions governing the import of products containing milk or milk products originating in, or consigned from China. The level of 2.5 mg/kg was considered as the appropriate level to distinguish between the unavoidable background presence of melamine and unacceptable adulteration and products shown to be non-compliant required destruction.

In light of a decrease in the number of notifications for unacceptable levels of melamine for food and feed products from China in the Rapid Alert System for Food and Feed (RASFF), Commission Regulation EC No. 2009/1135 [11] entered into force on 16 December 2009. The Regulation repeals and replaces Decision 2008/798/EC and reduces the intensity of physical checks to be carried out. The level of 2.5 mg/kg for melamine continues to remain appropriate and products that contain a higher level of melamine should be safely disposed of.

2. TEST MATERIAL

2.1. Preparation

The test material was prepared by a laboratory contracted to do so by FAPAS[®].

Approximately 7 kg of chocolate known to contain melamine was placed in a container, melted using a water bath at 50°C and stirred using a paddle stirrer for 30 minutes. The melted chocolate was then spread out over foil covered trays and left to cool at 4°C. Once set, the chocolate was grated and a portion was taken for screening. After screening the grated chocolate was cryogenically milled to a powder.

Individual sub-samples (≈50 g) were dispensed into numbered foil sachets and stored at -20°C prior to distribution.

2.2. Homogeneity

Ten randomly selected test materials were analysed in duplicate for melamine by a laboratory contracted to do so by FAPAS[®]. Replicate two from sample pair five is missing due to a failed extract that could not be analysed. This pair of data was therefore removed from the homogeneity calculations. The statistical tests initially check the data for any widely discrepant pairs using Cochran's test and if found such data are removed. Thereafter, the

remaining data are subject to analysis of variance (ANOVA) to estimate the sampling and analytical variances.

The results, together with their statistical evaluation [12], are given in APPENDIX I. The data show sufficient homogeneity, and are not included in the subsequent calculation of the assigned value.

2.3. Distribution

The dispatch date was 15 September 2009. Each participant received an individually numbered chocolate test material packed in an insulated box, together with cool blocks to control temperature fluctuations whilst in transit. A covering letter, instructions for the electronic submission of results and methods and a form for the submission of results for use by those participants without access to the Internet, were included in the package.

3. RESULTS

Participants were requested to report their results for melamine in mg/kg in the test material. Participants were asked to state which type of recovery procedure was used, their recovery (%) (if appropriate) and whether or not the recovery was applied to the result. If an internal standard had been used participants were asked to specify the name of the standard. Seventy two participants submitted results before the closing date for this test, 28 October 2009.

Each participant was given a laboratory number, assigned in order of receipt of results. The reported concentrations for melamine are given in Table 1.

The analytical methods used by each participant are summarised in APPENDIX II.

4. STATISTICAL EVALUATION OF RESULTS

The object of the statistical procedure employed is to obtain a simple and transparent result, which the participant and other interested parties can readily appreciate. The procedure follows that recommended in the IUPAC/ISO/AOAC International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [13].

4.1. Calculation of the Assigned Value, \hat{X}

The assigned value, \hat{X} , i.e. the best estimate of the true concentration of each analyte, was set as the consensus of the results submitted by participants. The procedure used to derive this consensus involved:

- Removing non valid data, i.e.:
 - i) results reported as approximately 10, 100 or 1000 x greater or smaller than the majority of submitted results (as these were considered to be reporting errors).
- Minimising the influence of outliers by the use of a robust statistical procedure to derive the mean [1].
- Assessing the standard uncertainty (u) of the robust mean.

$$u = \frac{\hat{\sigma}}{\sqrt{n}}$$

where $\hat{\sigma}$ = the standard deviation of the robust mean
 NB this is NOT the target standard deviation for the test (σ_p)

and n = the number of data points used to calculate the robust mean.

- Considering the normality, or otherwise (Kolmogorov-Smirnov test), of the distribution of results.

This procedure was straightforward for melamine. The consensus robust mean was considered the most appropriate measure of central tendency of participants' results and was therefore used to set the assigned value.

The robust mean used to set the assigned value, together with u , n and $\hat{\sigma}$ is shown in Table 2.

4.2. Target Standard Deviations for the Test, σ_p

The value of σ_p determines the limits of satisfactory performance in a FAPAS[®] proficiency test. It is set at a value that reflects best practice for the analyses in question. The standard deviation of reproducibility found in collaborative trials is generally considered to be an appropriate indicator of the best agreement that can be obtained between laboratories. However, not all analyses have been characterised in this manner. In such cases the predictive models of the appropriate form of the Horwitz equation [3] are usually valuable indicators of best practice.

This is the first proficiency test from FAPAS[®] to contain melamine in food and as no appropriate collaborative trial data are available for melamine σ_p was derived from the appropriate form of the Horwitz equation [3]. This equation predicts a standard deviation from a given concentration, c , and requires c to be expressed as a dimensionless mass ratio, e.g. 1 ppm $\equiv 10^{-6}$ or % $\equiv 10^{-2}$. It follows therefore that to express the dimensionless standard deviation predicted by the equation in the original concentration units it must be divided by the relevant mass ratio:

- i) for analyte concentrations <120 ppb

$$\sigma_p = \frac{0.22c}{mr}$$

- ii) for analyte concentrations ≥ 120 ppb and $\leq 13.8\%$

$$\sigma_p = \frac{0.02c^{0.8495}}{mr}$$

- iii) for analyte concentrations >13.8%

$$\sigma_p = \frac{0.01c^{0.5}}{mr}$$

where, in all three cases, c = concentration, i.e. the assigned value, \hat{X} , expressed as a dimensionless mass ratio, e.g. 1 ppm $\equiv 10^{-6}$ or % $\equiv 10^{-2}$

and mr = dimensionless mass ratio, e.g. 1 ppm $\equiv 10^{-6}$ or % $\equiv 10^{-2}$.

The value for σ_p used to calculate z-scores from the reported results for melamine in this proficiency test is given in Table 2.

4.3. Individual z-Scores

Participants' z-scores were calculated as:

$$z = \frac{(x - \hat{X})}{\sigma_p}$$

where x = participant's reported result,

\hat{X} = the assigned value

and σ_p = target standard deviation.

Participants' z-scores for melamine are given in Table 1 and are shown as a histogram in Figure 1.

The number and percentage of z-scores in the satisfactory range for melamine, $|z| \leq 2$, are given in Table 3.

It is possible for the z-scores published in this report to differ slightly from the z-score that can be calculated using the formula given above. These differences arise from the necessary rounding of the actual assigned value and target standard deviation prior to their publication in Table 2.

5. REFERENCES

- 1 Analytical Methods Committee, 1989, Robust Statistics – How not to reject outliers Part 1. Basic Concepts, *Analyst*, **114**, 1693-1697.
- 2 Lowthian, P. J. and Thompson, M., 2002, Bump-hunting for the proficiency tester – searching for multimodality, *Analyst*, **127**, 1359-1364.
- 3 Thompson, M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, *Analyst*, **125**, 385-386.
- 4 ISO/IEC 17025:2005, General requirements for the competence of testing and calibration laboratories.
- 5 Regulation (EC) 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, *Official Journal*, **L 165**, 30/04/2004, 0001-0141.
- 6 FAPAS[®], 2009, Protocol for Proficiency Testing Schemes, Part 1 – Common Principles, Revision 2009, Version 1, Issued November 2009.
- 7 FAPAS[®], 2009, Protocol for Proficiency Testing Schemes, Part 2 – FAPAS[®], Revision 2009, Version 1, Issued November 2009.
- 8 World Health Organization, 2009, Toxicological and health aspects of melamine and cyanuric acid: report of a WHO expert meeting in collaboration with FAO, supported by Health Canada, Ottawa, Canada, 1–4 December 2008, WHO Press, 001-066.
- 9 Commission Decision 2008/798/EC of 14 October 2008 imposing special conditions governing the import of products containing milk or milk products originating in or consigned from China, and repealing Commission Decision 2008/757/EC, *Official Journal*, **L 273**, 15/10/2008, 0018-0020.
- 10 Commission Decision 2008/921/EC of 9 December 2008 amending Decision 2008/798/EC, *Official Journal*, **L 331**, 15/10/2008, 0019-0020.
- 11 Regulation (EC) 2009/1135/EC of 25 November 2009 imposing special conditions governing the import of certain products originating in or consigned from China, and repealing Commission Decision 2008/798/EC, *Official Journal*, **L 311**, 26/11/2009, 0003-0005.
- 12 Fearn, T. and Thompson, M., 2001, A new test for sufficient homogeneity, *Analyst*, **126**, 1414-1417.
- 13 Thompson, M., Ellison, S.L.R. and Wood, R., 2006, The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, *Pure Appl. Chem.*, **78**, No. 1, 145-196.

Table 1: Results and z-Scores for Melamine in Chocolate Test Material

laboratory number	analyte				z-score
	melamine				
	assigned value 5.69 mg/kg				
	result mg/kg	internal standard used (Y/N) if Y, specify name	if appropriate state % recovery & if applied (Y/N)		
001	6.463	Y	Y		1.1
002	2.1	Y,Melamine 13C	N		-5.1
003	2.6	N	N		-4.4
004	5.5	13C3, 15N3-Melamine	99% No		-0.3
005	† 5.1	Melamin-13C3-15N3	49%		-0.8
006	11.1	Yes- melamine 3C13.3N15	95% -No		7.7
007	6.09	N	88.1 N		0.6
008	4.9	Y, Melamine 13C3 15N3	N		-1.1
009	11.4	Melamin-15N3	N		8.1
010	5.42	(Y) 13C3-15N3-Melamin	109.8 (Y)		-0.4
011	5.10	N	89 N		-0.8
012	7.148	N	121% No		2.1
013	4.4	y, melamine 13C3 15N3	40%, y		-1.8
014	7.31	N	107 %, N		2.3
015	‡ 7.62	N	N		2.8
016	5.65	13C3-Melanin	95%, Y		-0.1
017	5.2	% Recovery	105% Y (Applied)		-0.7
018	4.23	2,6 diamino-4-chloro pyrimidine	106% N		-2.1
019	5.72	Y- 13C melamine	N		0.0
020	5.94	13C3, 15N3-Melamine	No		0.4

Results and comments are presented as reported by participants.
z-Scores outside the satisfactory range i.e. $|z| > 2$ are shown in **bold**.

Participants' comments:

† = Result was corrected by the Recovery of 49% of the internal standard Melamin-13C3-15N3.

‡ = not corrected for recovery

Table 1 (continued): Results and z-Scores for Melamine in Chocolate Test Material

laboratory number	analyte				
	melamine assigned value 5.69 mg/kg				
		result mg/kg	internal standard used (Y/N) if Y, specify name	if appropriate state % recovery & if applied (Y/N)	z-score
021	▲	26.76	N	N	30.1
022		6.92	N	88% (N)	1.8
023		7.31	N	N	2.3
024	♠	5.79	Y, 13C315N3-melamine	Y	0.1
025		9.82	N	68 % / Y	5.9
026		4.148	N	N	-2.2
027		5.53	melamine 13C3, 99%; amino-15N3, 98%	107%; N	-0.2
028		6.07	Y, Melamine 13C3 15N3	105% (N)	0.5
029		5.92	Melamine-13C3	N	0.3
030		3.06	N	N	-3.8
031		5.9	Y,Melamine(13C3, 99%,Amino-15N3,98%	100 % (N)	0.3
032		0.48	2,6-diamino-4-chloropyrimidine	N	-7.4
033		7.89	N	N	3.1
034		6.04	Y, Melamine-15N3	N	0.5
035		5.2	N	99.9%, N	-0.7
036		5.76	Y	Y	0.1
037		12.2	N	N	9.3
038	♪	6.2	N	N	0.7
039		5.61	cyromazine	N	-0.1
040	†	6.29	Y, C13N15-Melamine	Not Applicable	0.9

Results and comments are presented as reported by participants.
z-Scores outside the satisfactory range i.e. $|z| > 2$ are shown in **bold**.

Participants' comments:

▲ = matrix match standard calibration

♠ = used matrix calibration curve

♪ = Method of standard addition are used

† = Matrix based Cals used

Table 1 (continued): Results and z-Scores for Melamine in Chocolate Test Material

laboratory number	analyte			
	melamine assigned value 5.69 mg/kg			
	result mg/kg	internal standard used (Y/N) if Y, specify name	if appropriate state % recovery & if applied (Y/N)	z-score
041	15.72	N	60% (Y)	14.3
042	▼ 5.74	N	N	0.1
043	5.56	N	91.2%&N	-0.2
044	6.07	Y,Melamine-15N3	97.1%,N	0.5
045	4.75	N	N	-1.3
046	4.90	N	98.0%	-1.1
047	4.38	Y, *C3H6*N3N3	N	-1.9
048	♣ 5.62	N	91.46% & Y	-0.1
049	4.80	y, 13C315N3-Melamin	113 %, n	-1.3
050	5	melamine(13C3,99%,15N3,98%)	N	-1.0
051	♪ 4.8	Yes, Melamine-triamine 15N3 ISOTEC	92,3% not applied	-1.3
052	5.88	N	90% (N)	0.3
053	6.8	Y, {13C3,15N3}-melamine	Y, 107%	1.6
054	3.8	13C3, amino-15N3 Melamine	N	-2.7
055	5.56	N	90.7&N	-0.2
056	7.10	N	N	2.0
057	3.0	13N	N	-3.8
058	4.4	N	89	-1.8
059	6.21	Y(13C3-melamine)	Y(100)	0.7
060	8.01	n	77	3.3

Results and comments are presented as reported by participants.
z-Scores outside the satisfactory range i.e. $|z| > 2$ are shown in **bold**.

Participants' comments:

▼ = recovery:97.5%

♣ = (it is not recovery corrected), recovery corrected result: 6.08mg/kg

♪ = Recovery was not applied to the result.

Table 1 (continued): Results and z-Scores for Melamine in Chocolate Test Material

laboratory number	analyte			
	melamine assigned value 5.69 mg/kg			
	result mg/kg	internal standard used (Y/N) if Y, specify name	if appropriate state % recovery & if applied (Y/N)	z-score
061	6.25	Y	N	0.8
062	4.14	N	N	-2.2
063	5.81	Y, 13C3-Melamine	N	0.2
064	4.3	N	104	-2.0
065	5.0	Y Melamine 3C13 3N15	N	-1.0
066	4.86	13C3-15N3 Melamine	N/A	-1.2
067	5.81	Y, Melamine 13C3	result was calculated based on stan	0.2
068	5.5	Y, 13C3-15N3-Melamine	114%, N	-0.3
069	2.76	Y (2,6-diamine-4-chloropyrimidine)	N	-4.2
070	3.51	N	82.2	-3.1
071	7.0	N0	No	1.9
072	▶ 5.7	Melamine 13C3,99%:Amino-15N3,98%)	N	0.0

Results and comments are presented as reported by participants.
z-Scores outside the satisfactory range i.e. $|z| > 2$ are shown in **bold**.

Participants' comments:

▶ = Signs of persistent sample inhomogeneity

Table 2: Assigned Values and Target Standard Deviations

analyte	assigned value, mg/kg				target standard deviation, mg/kg	
	data points, n	robust mean, \hat{X}	robust standard deviation, $\hat{\sigma}$	uncertainty, u	derived from	σ_p
melamine	71	5.69	1.39	0.165	Horwitz*	0.701

*See page 7 for appropriate form of the Horwitz equation.

Table 3: Number and Percentage of Satisfactory z-Scores

analyte	number of satisfactory scores, $ z \leq 2$	total number of scores	satisfactory, %
melamine	49	72	68

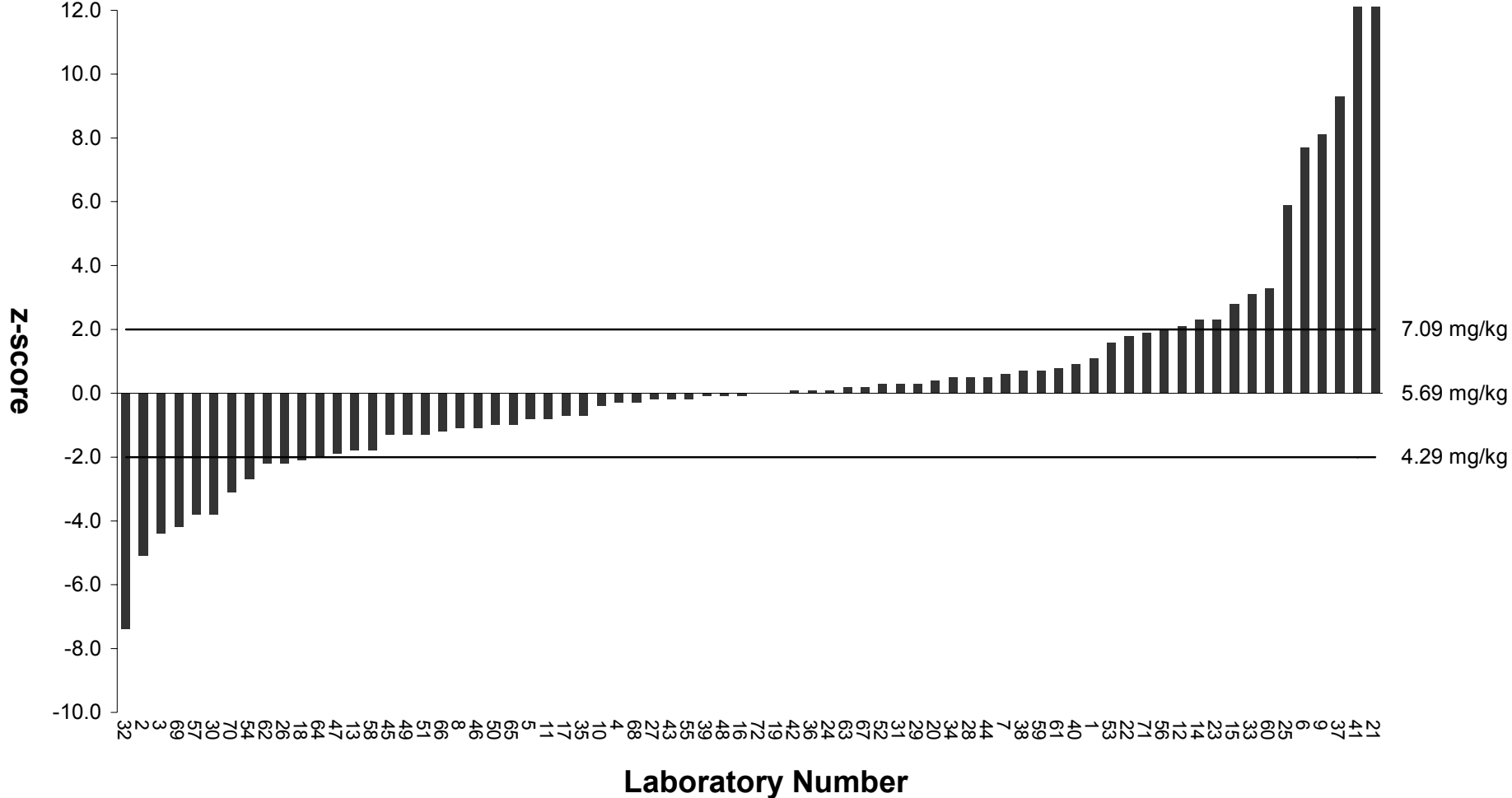


Figure 1: z-Scores for Melamine (5.69 mg/kg) in Chocolate Test Material

APPENDIX I: Homogeneity Data for Melamine in Chocolate Test Material

sample identity	analyte	
	melamine mg/kg	
	replicate 1	replicate 2
1	11.07	11.04
2	10.30	10.26
3	11.90	13.61
4	11.18	10.28
5	10.95	no result
6	12.68	10.30
7	11.08	10.20
8	11.27	10.56
9	10.12	9.84
10	11.44	12.26
mean, <i>n</i>	11.08	18
origin of target standard deviation (σ_p)	Horwitz*	original
absolute target sd (σ_p) & as RSD%	1.23	11.1
s_{an}		0.797
s_{sam}^2		0.403
σ_{all}^2		0.137
<i>critical</i>		0.971
$s_{sam}^2 < critical?$		ACCEPT

*See page 7 for appropriate form of the Horwitz equation.

APPENDIX II: Analytical Methods Used by Participants

Notes:

- 1) Participants' methods are tabulated according to the information submitted electronically, but some responses may have been combined or edited for clarity.

Accredited Method Used	laboratory number
yes	005 007 008 013 016 020 026 027 029 035 036 037 040 041 042 043 044 048 050 053 054 055 059 061 065 068
no	002 003 004 006 009 010 012 014 015 017 021 022 023 024 028 030 032 033 034 038 039 046 047 049 051 052 060 062 063 064 066 067 069 070 071 072

Reference	laboratory number
Agilent Techn. Application HPLC-UV	014
Analytica Chemica Acta 2009 649 91-97	059
Application -No.304920, Macherey-Nagel 2009	029
Application note - Applied Biosystems	009 053
Applied Biosystem protocol for melamine analysis 2008	052
China National Standard GB/T 22388-2008, Determination of Melamine in Raw Milk and Dairy Products	020 035 042 043 044 055
CNA	002
FDA	003 032
FDA 2007	022 060
U.S. FDA, LIB No. 4396 2007	021 025 027 061
USFDA Laboratory Information Bulletin, No. 4421 2008	013 047 065
USFDA Laboratory Information Bulletin No 4422, Oct 2008	006 024 028 065 066

Reference (continued)	laboratory number
FERN	011
Korea Food Regulation 2009	026
Korea Food Code 2009 1 10-7-50	048
Rapid Communications in Mass Spectrometry 2008 22 3624-3632	016
RASFF NEWS 2007 07-369	037
USDA	041
USDA 2008 23	036
Waters Application Note 2008	038 051 063

Sample Amount Used for Analysis (g)	laboratory number
<1	004 005 006 008 012 020 028 034 037 049 059 060 063
≥1 - <2	003 011 014 016 022 023 026 027 029 031 032 036 038 040 044 046 047 048 052 058 061 066 068 072
≥2 - <5	002 007 010 013 015 021 024 025 033 035 042 043 050 051 054 055 062 065 067 069
≥5 - <10	009 041 064 071

Internal Standard Added?	laboratory number
yes	002 004 005 006 008 009 010 013 016 020 024 027 028 029 031 032 034 036 038 039 040 044 047 049 050 051 053 054 059 061 063 065 066 067 068 069 072
no	003 007 011 012 014 015 017 021 022 023 025 026 030 033 035 037 041 042 043 046 048 052 055 058 060 062 064 070 071

If Yes, Please Specify the Internal Standard Used	laboratory number
melamine	047
cyromazine	039
¹⁵ N ₃ -melamine	009 034 044 051
¹³ C ₃ -melamine	002 016 029 061 063 067
¹³ C ₃ - ¹⁵ N ₃ -melamine	004 005 006 008 010 020 024 027 028 031 036 038 040 049 050 053 054 065 066 068 072
2,6-diamine-4-chloropyrimidine	032 069

Point at Which Internal Standard was Added	laboratory number
prior to extraction	002 005 006 008 009 010 013 016 020 024 028 029 031 034 036 039 040 044 047 049 050 051 054 061 063 067 068 069 072
after extraction	004 066
prior to clean up	027 065
derivatisation step	032
before final step	059
prior to instrument measurement	053
prior to injection to LC-MS	038

Extraction Solvent	laboratory number
acetic acid	005 024
acetonitrile	002 003 004 005 008 010 011 012 014 021 022 023 025 026 027 028 031 032 033 034 035 036 037 039 040 046 047 048 049 050 051 052 053 060 066 069 072
ammonium formate buffer	034
dichloromethane	021 054 061
diethylamine	008 032 037 069
formic acid	013 059 065 067 068
hexane	005
hydrochloric acid	029 033
methanol	016 041 068

Extraction Solvent (continued)	laboratory number
perchloric acid	038 063 064
phosphate buffer	055
trichloroacetic acid	006 014 020 034 035 039 042 043 044 051 070
trifluoroacetic acid	007 017 062
water	003 004 008 009 010 011 012 014 016 017 020 021 022 023 025 026 027 028 031 032 036 037 038 039 040 041 046 047 049 053 055 058 065 066 068 069 071

Extraction Technique Used	laboratory number
blending	031
cold solvent extraction at atmospheric pressure	003 005 006 008 010 011 012 013 016 017 020 022 023 026 027 028 032 033 035 037 038 039 040 044 047 049 050 051 053 055 058 059 062 064 065 066 067 068 070 072
hot solvent extraction at atmospheric pressure (e.g. soxhlet)	009 014 029 042 046 071
liquid-liquid extraction	036
shake, blend with solvent at 50 C	007
shaking	063
shaking, vortex and centrifuge	021
1 min Ultra Turrax/centrifugation	025
sonication, centrifuge	048
ultrasonic bath 70°C, 30 min	004
ultrasonication	002 069
vortex and shaker	024

Extraction pH Adjusted	laboratory number
yes	034 036 040 058 065
no	002 004 005 006 007 008 009 010 011 012 013 014 016 017 020 021 022 023 024 025 026 027 028 029 031 032 033 035 037 038 039 041 042 043 044 046 047 048 049 050 051 052 053 054 055 057 059 060 061 062 063 064 066 067 068 069 070 071 072

Method Included a Sample Drying Step?	laboratory number
yes	011 021 022 027 028 029 036 037 038 040 041 046 049 051 066 069
no	002 003 004 005 006 007 008 009 010 012 013 014 016 017 020 023 024 025 026 031 032 033 034 035 039 042 043 044 047 048 050 052 053 054 055 058 059 060 061 062 063 064 065 067 068 070 071 072

If 'yes' Please Specify	laboratory number
after clean up the eluate is dried in a heating block at 55+/-5 deg C	066
evaporate to dryness at 70°C	037
cartridge was dried under vacuum after SPE	027
drying under nitrogen before analysis	011 021 046 051
40°C nitrogen	049
50°C nitrogen	028
after SPE with nitrogen, 55°C	029
nitrogen stream, 60°C	069
rotary vacuum evaporation	022

Sample Clean-up Technique	laboratory number
cation exchange resin (cartridge)	048
extraction	042 059
filter	002 004 009 044 060 069
liquid/liquid extraction	005 034 052 053
pH adjusted	025
precipitate proteins 2 – 4°C	014
protein denaturation, centrifugation	004
silica column	050
solid phase extraction (SPE) (column/cartridge)	003 006 007 010 011 017 020 021 022 024 025 026 027 028 029 033 034 035 036 038 039 040 041 043 044 046 047 049 051 054 055 057 058 061 062 063 064 065 066 067 068 070 072
none	008 012 013 016 031 032 071

SPE Column Type	laboratory number
Bond Elut AccuCAT	050
C18	017
Chromabond HR-XC	029
Envi-chrom P/Strata-X	025 026 054 068
Isolute Multi-Mode M-M	034
MCX	020 040 041 043 044 061 064 066
Waters OASIS MCX	003 028 036 042 051 065
PCX	055
SAX	058
SCX	006 007 011 024 035 039 046 049 057 062 070 072
Strata Melamine	067

Certified Standards Used	laboratory number
yes	002 004 005 008 010 011 012 013 016 021 022 026 027 028 029 033 035 037 040 041 042 043 044 046 048 049 050 051 052 053 055 057 058 059 063 064 065 066 067 069 070 072
no	003 006 007 009 014 015 017 020 024 025 031 032 034 038 039 047 054 061 068 071

Calibrations	laboratory number
matrix-matched	003 008 021 023 033 039 040 052 053 061 063
multi-level	004 005 006 007 010 011 012 013 014 015 016 017 020 024 025 026 027 028 031 034 035 036 037 040 041 044 047 048 049 050 051 054 055 059 064 065 066 068 070 071 072
single-level	009 032 042 043 060
solvent	004 009 010 011 012 013 016 027 044 046 047 051 065
standard addition	002 022 029 038 058 062 067 069

Source of Standards	laboratory number
Abraxis	071
Acros	010
Aldrich	028
Cambridge Isotope Lab. Inc.	002 005 008 049 059
ChemService	012 064
ChromaDex	020 060
Dr Ehrenstorfer	004 011 023 035 044 046 053 069 072
Fluka	022 029 033 042 046 047 049 055 061 063 065
LGC Promochem	004 010
Merck	032
Riedel De Haan	031
Sigma/Aldrich	006 007 008 009 013 015 016 017 024 025 026 027 034 035 036 037 038 039 040 041 043 048 051 052 054 058 062 066
Supelco	070
Wako	050
Wellington	068
Witega Laboratorien	063

Quoted Percentage Recovery was Measured in Same Analytical Batch as the Test Material	laboratory number
yes	004 006 007 008 010 011 012 013 016 017 020 022 024 025 027 028 029 031 033 035 036 039 041 042 043 044 048 049 051 052 053 058 061 064 065 070 072
no	002 005 014 026 032 034 037 038 046 050 055 059 062 063 069 071

If Measured in this Batch, at What Stage was the Spike Added	laboratory number
prior to extraction	003 004 006 007 008 010 011 012 013 014 016 017 020 022 024 025 026 028 029 031 033 035 036 041 042 043 044 046 048 049 050 051 052 053 058 062 064 065 070 072
prior to clean up	002 027 039

Level of Spike (mg/kg)	laboratory number
<1	027 044 061
≥1 - <5	002 003 005 006 007 008 011 012 013 014 016 017 020 022 026 028 035 036 038 042 046 048 053 058 061 062 064 072
≥5 - <10	004 010 016 024 025 029 031 033 038 039 041 043 049 050 051 052 070
≥10 - <15	029 049 065
≥15 - <20	067
≥25 - <30	067
≥30 - <40	067

Composition of Blank Commodity Used for Spiking	laboratory number
test material provided	002 004 007 010 013 016 017 024 026 029 035 036 038 042 043 048 050 055 058 061 065 070 071
chocolate	008 011 022 034 040 044 051 052 062 063 064
chocolate powder	020 053
cocoa and milk powder	031
milk powder	012
own blank	028 033 046 072
water	009

Determination	laboratory number
ELISA	071
GC	020 032 037 039 068 069
HPLC	002 004 005 006 007 008 009 010 011 012 013 014 016 017 022 024 025 026 028 029 033 034 035 036 038 040 041 042 043 044 046 048 049 050 051 052 053 055 059 061 062 063 065 067 070 072
UPLC	064

GC Column Type	laboratory number
capillary	020 032 037 039 060 068

GC Column Packing	laboratory number
95% methyl 5% phenyl polysiloxane	020 032 037 039 060 068 069

GC Injection Volume (µL)	laboratory number
≥1 - <2	020 032 037 039 060 068 069

GC Injection Mode	laboratory number
split	039
splitless	020 032 037 060 068 069

GC Derivatisation Reagent Used	laboratory number
BSTFA	020 037 069
HMDS	032
pyridine	037
trimethylchlorosilane	032 069

GC Detector	laboratory number
MSD	020 032 037 039 068 069
MS-MS	060

HPLC Conditions	laboratory number
normal phase	002 006 008 014 022 029 031 041 042 044 047 050 053 058 059 064 072
reverse phase	004 005 007 009 010 011 015 017 024 026 027 033 035 036 038 043 046 048 049 051 052 062 063 065 070

HPLC Column Packing	laboratory number
C18	007 009 013 017 024 026 030 033 034 035 043 046 048 064 070 072
C18 X-Terra type	055
C8	014
endcapped	005 035 044
HILIC	004 006 007 008 012 016 025 027 028 029 031 036 038 040 041 044 047 051 052 053 063 065 066
ion exchange	033
SCX	042
NH ₂	010 011
silica	058
TSK-gel-Amide-80	049

Used HPLC Guard Column	laboratory number
yes	003 005 006 007 012 013 014 016 017 022 024 029 031 035 038 041 046 048 049 050 052 053 055 058 063 064 065 070
no	002 004 008 009 010 011 021 025 026 027 028 033 034 036 040 042 044 047 051 057 059 062 072

Mobile Phase Programme	laboratory number
isocratic	006 007 011 017 021 022 023 024 026 031 033 034 035 038 041 042 046 047 048 062 063 066 070
gradient	002 003 004 005 008 009 010 012 013 014 016 025 027 028 029 036 040 044 049 050 051 052 053 055 057 058 059 064 065 072

Mobile Phase Components	laboratory number
acetate	002 012 022 051 052 053 064
acetic acid	010
acetonitrile	002 003 004 005 006 007 010 011 012 013 016 017 021 022 023 024 025 027 028 029 033 035 038 040 041 044 047 048 049 050 051 052 053 058 059 062 063 065 066 070 072
ammonium acetate	005 021 024 025 027 028 047 049 062 063 066
ammonium formate	004 006 007 017 029 034 041 065
etnionine	042
formic acid	003 004 007 016 036 038 040 059
ion pair agent	035
methanol	005 009 013 014
perchloric acid	055
phosphate	042 070
water	002 003 004 005 009 010 011 013 014 016 021 023 025 026 027 031 038 040 044 051 052 053 059 062 063 065 066

HPLC Column Temperature (°C)	laboratory number
ambient	006 007 011 012 014 016 021 026 028 034 042 043 044 055 058 070
>ambient - <50	002 003 004 005 008 009 010 013 017 022 023 024 025 027 029 031 033 035 038 040 041 046 047 048 049 050 051 052 053 059 062 063 064 065 072

HPLC Injection Volume (µL)	laboratory number
<5	010 011 023 029 044 046 064 072
≥5 - <10	006 008 021 038 040 041 048 049 050 051 059 063 065 066
≥10 - <25	002 003 004 005 009 012 014 016 022 025 026 027 028 031 033 035 036 042 043 047 052 053 055 058 062
≥25 - <50	024
≥50 - <100	007 013 017 070
≥100 - <150	034

Mobile Phase Flow Rate (mL/min)	laboratory number
<0.25	002 006 010 011 024 029 036 043 046 050
≥0.25 - <0.75	003 004 005 007 008 009 013 014 016 017 021 022 023 025 027 028 031 033 034 038 040 041 044 047 049 051 052 053 058 059 062 063 064 065 066 072
≥0.75 - <1.25	012 026 035 042 048 055 070

HPLC Detector Type	laboratory number
Diode Array Detector	017 026 035 042 046 048
MS-MS	003 004 005 006 008 009 010 011 012 013 015 016 021 022 023 024 025 027 028 029 031 033 034 036 038 040 041 044 047 049 050 051 052 053 058 059 062 063 064 065 066 072
UV	014 055 070
UV/Vis	007 043

Wavelength (Absorbance)(nm)	laboratory number
208	007 017
214	007
215	070
235	043 046
240	014 026 035 042 048 055

Acquisition Mode	laboratory number
ESI	022
ESI+	010 016 049 059 062
positive	006 011 021 024 040 044
SRM	031
MRM	004 008 013 023 025 028 029 033 040 044 047 052 053 059 063

Transitions Monitored	laboratory number
127>43	022 044
127>57	051
127>58	051
127>60	025 027
127>67	062
127>68	003 004 006 008 011 013 016 021 022 024 025 027 029 031 033 034 038 040 044 047 052 063 065
127>71	051
127>84	062
127>85	002 003 004 006 008 011 013 016 021 022 024 025 027 029 031 033 034 036 038 040 044 047 049 051 052 063 065
127>110	034
130>70	034
130>87	034 044
133>45	004
133>62	005
133>71	005 047
133>89	047

Lower Reporting Limit (mg/kg)	laboratory number
<0.5	007
0.005	052
0.01	029 031
0.012	027
0.02	048
0.04	072
0.05	006 022 023 033 034 040 041 050 065
0.1	004 013 021 028 038 053
0.2	015 016 017 054
0.3	036
0.5	011 032 044 049 062 070
1	009 051
1.5	063
2	035 042

Analysis Included Cyanuric Acid Determination?	laboratory number
yes	004 012 013 016 053 065
no	002 003 005 006 007 008 009 010 011 014 015 017 020 021 022 023 024 025 026 027 028 029 031 032 033 034 035 036 038 039 040 041 042 043 044 047 048 049 050 051 052 054 055 059 062 063 064 068 069 070 072
Time Between Extraction and Determination (hours)	laboratory number
0 (immediate)	006 010 023 025 031 038 044 048 052 059 070
<12	005 007 008 009 011 013 014 016 017 020 021 022 027 029 032 033 034 035 036 040 041 042 043 046 047 049 050 051 055 063 064 065 068 069 072
<24	003 004 012 024 026 053 062
≥24	028
Reagent the Final Extracts were Dissolved in	laboratory number
acetate buffer	064
acetic acid	010
acetonitrile	002 003 004 005 007 010 013 014 016 021 024 025 027 028 029 033 034 036 040 041 044 049 051 054 062 063 064 065
ammonia	006
ammonium acetate	021 024 027 028 033 050 062
ammonium formate	008 029 034 041
BSTFA	020
carbinol	042
DEA	003
formic acid	004 065
heptane	032

Reagent the Final Extracts were Dissolved in? (continued)	laboratory number
HPLC eluent	047
methanol	006
mobile phase	021 035 038 043 046 066 070
pyridine	020 068 069
trichloroacetic acid	014
water	002 009 010 011 014 021 025 036 040 042 044 048 049 051 054 062 063

APPENDIX III: FAPAS[®] SecureWeb, Reports and Protocol

1. FAPAS[®] SECUREWEB

Access to the secure area of our web site is only available to participants in our proficiency tests. Please contact us if you require a UserID and Password. FAPAS[®] SecureWeb allows participants to:

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3. PROTOCOL

The Protocols [6, 7] set out how FAPAS[®] is organised. Copies can be downloaded from our website.

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Participants with any comments or concerns about this proficiency test should contact:

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