DLA
Dienstleistung
Lebensmittel
Analytik GbR

Evaluation Report
proficiency test

19/2014

Melamine

in Drink Powder with Milk Powder and Cocoa

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Content

1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed, cosmetics and food contact materials. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the validity of the particular testing method.
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009 (6).

2. Realisation

2.1 Test material

The test material is a mixture of two customary in commerce cocoa drink powders from European manufacturers. The material was spiked with melamine. After homogenization the first spiked level was again supplemented with the drink powder mixture and homogenized. This procedure was repeated until the desired spike level was reached.
Afterwards the test material was packaged lightproof to approximately 25 gram each. The portions were numbered chronologically.

Table 1: Composition of DLA-Sample

Ingredients	**Content**
Cocoa Drinking Powder Ingredients: Sugar, glucose, cocoa powder (defatted 20%), emulsifier: lecithins, salt Nutrition values per 100g: Energy 1600 kJ / 380 kcal, protein 4,5 g, carbohydrates 80 g, fat 13 g	80 g / 100 g
Cocoa Drinking Powder with Milk Powder Ingredients: Whey Powder (39%), sugar, cocoa powder (15%), cocoa powder defatted (5%), coconut oil hydra-ted, skimmed milk powder (2,4%), salt, stabilizer E339, aroma Nutrition values per 100g: Energy 1627 kJ / 386 kcal, protein 9,3 g, carbohydrates 65 g, fat 6,9 g	20 g / 100 g
Melamine Chemical p.A.	23 mg/kg

2.1.1 Homogeneity

For melamine the calculation of the repeatability standard deviation of the participants results was suitable as an indicator of homogeneity in the present PT. With 7,6 % it was in the range of the criterium for the relative repeatability standard deviation of 15% according to ISO/TS 15495 (13).
The repeatability standard deviation of the participants for melamine is given in the documentation.

Additionally in the documentation the portion numbers are graphically assigned to the results of melamine.

2.2 Test

Two portions of test material were sent to every participating laboratory in the 42nd week of 2014. The testing method was optional. The tests should be finished at 28th november 2014.

2.3 Submission of results

The participants submitted their results in standard forms, which have been sent by email or were available on our website.
The finally calculated concentration of melamine as an average of a duplicate determination of both numbered samples was used for the statistical evaluation. Queried and documented were single results, recovery and the testing methods used.
One participant submitted no results. All other participants submitted the results in time.

3. Evaluation

3.1 Assigned value

Because the analysed material was no certified reference material the robust mean of the submitted results was used as assigned value X (6). The distribution of submitted results showed no hint for bimodal distribution or other reasons for a higher variability.

3.2 Standard deviation

For comparison to the target standard deviation a robust standard deviation (S^x) was calculated (6).

3.3 Outliers

Statistical outliers were determined by Mandel´s-H-Statistic for 95% significance niveau (5). Detected outliers were stated for information only, when z-score was < -2 or > 2.

3.4 Target standard deviation

The target standard deviation of the assigned value is determined according to the following methods.

In general the Horwitz target standard deviation is suitable for the statistical evaluation of interlaboratory tests where different analytical methods are applied. The standard deviation from precision experiments are derived from proficiency tests where a specific analytical method is mandatory.

In the present proficiency test for melamine the target standard deviation according to the general model (Horwitz) was applied.

3.4.1 General model (Horwitz)

The relative target standard deviation in % of the assigned value is derived from following equation (Horwitz)

$$\hat{\sigma}_{(\%)} = 2^{(1-0,5\log X)}$$

From the result the target standard deviation is calculated

$$\hat{\sigma} = X * \hat{\sigma}_{(\%)} / 100.$$

3.4.2 Value by precision experiment

Using the reproducibility standard deviation σ_R and the repeatability standard deviation σ_r of a precision experiment the between-laboratories standard deviation can be calculated σ_L :

$$\sigma_L = \sqrt{(\sigma_R^2 - \sigma_r^2)} \quad .$$

And then, using the number of replicate measurements n, each participant is to perform, the target standard deviation for proficiency assessment is calculated :

$$\hat{\sigma} = \sqrt{(\sigma_L^2 + (\sigma_r^2/n))} \quad .$$

According to the guidelines for the quantitative determination of melamine and cyanuric acid by LC-MS/MS the repeatability standard deviations for melamine were 6% and 12% and the within-laboratory reproducibilities were 13% and 15% for cow's milk and powdered infant formula respectively (13).

For further information the following selection of precision data of published applications for the determination of melamine using different methods is given:

The coefficients of variation (inter- and intra-assay) for the methods of HPLC-DAD, LC-MS and ELISA were each < 9,31% for supplemented feed (Kim et al. 2008). The study was performed by a working group applying the 3 different methods.
The relative standard deviation of a LC-MS-Method for the determination of melamine in fish and crustacean was 21,5% (n=121) (Andersen et al. 2008).
The recovery rates for different matrices were between 74% and 152% for the GC-MS FDA-Method (USFDA 2007).

3.4.3 Value by perception

The target standard deviation for proficiency assessment can be set at a value that corresponds to the level of performance that the coordinator would wish laboratories to be able to achieve (6).

For the evaluation in the present proficiency testing the model according to Horwitz was applied. With the normal relative target standard deviation the valuation was limited (quotients $S^x/\hat{\sigma}$ > 2 and $u_X/\hat{\sigma}$ > 0,3)). **Therefore the evaluation of participants' results was done using z'-values and the target standard deviation $\hat{\sigma}$'** (s. 3.6 and 3.8).

3.5 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation ($\hat{\sigma}$) the result (x) of the participant is deviating from the assigned value (X) (6).
Participants' z-scores were derived as:

$$z = (x - X) / \hat{\sigma} \ ;$$

the requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z \leq 2 \ .$$

3.6 z'-Score

The z'-score can be used for the valuation of the results of the participants, in cases the standard uncertainty has to be considered (s. 3.8). The z'-score represents the relation of the deviation of the result (x) of the participant from the respective assigned value (X) to the square root of quadrat sum of the target standard deviation ($\hat{\sigma}$) and the standard uncertainty (Ux) (6).

Participants' z'-scores are derived as:

$$z' = (x - X) / \sqrt{\hat{\sigma}^2 + u_X^2}$$

In the following we define the denominator $\sqrt{\hat{\sigma}^2 + u_X^2}$ as the target standard deviation $\hat{\sigma}'$.

The requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z' \leq 2 \ .$$

3.7 Quotient $S^x/\hat{\sigma}$

Following the Horrat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation and target standard deviation does not exceed the value of 2.
A value > 2 means an insufficient precision, i.e. the analytical method is too variable, or the variation between the test participants is higher than estimated. Thus the comparability of the results is not given (11).
In the present proficiency tests the quotient $S^x/\hat{\sigma}$ for melamine was 3,9. Therefore the evaluation was done according to 3.6 z'-scores. The quotient $S^x/\hat{\sigma}'$ was then 2,0.

3.8 Standard uncertainty

The assigned value X has a standard uncertainty u_X that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories and perhaps on other factors. The standard uncertainty u_X for this PT is calculated as follows (6).

$$u_X = 1{,}25 * S^x/\sqrt{(p)}$$

If $u_X \leq 0{,}3 * \hat{\sigma}$ the standard uncertainty of the assigned value needs not to be included in the interpretation of the results of the PT (6).

In the present proficiency test for melamine the quotient $U_x/\hat{\sigma}$ was 1,6. Therefore for the valuation of the participants' results the z'-Score considering the standard uncertainty was used (s. 3.6).

4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.
In the upper table the characteristics are listed:

Statistic Data
Number of results
Number of outliers
Mean
Median
Robust mean (X)
Robust standard deviation (S^x)
Target range:
Target standard deviation for information
Target standard deviation $\hat{\sigma}$ '
lower limit of target range (X - 2 $\hat{\sigma}$) or (X - 2 $\hat{\sigma}$ ') *
upper limit of target range (X + 2 $\hat{\sigma}$) or (X + 2 $\hat{\sigma}$ ') *
Quotient $S^x/\hat{\sigma}$ '
Standard uncertainty u_X
Quotient $u_X/\hat{\sigma}$ '
Number of results in the target range

* Target range is calculated with z-score or z'-score

In the lower table -laboratories- the individual results of the participating laboratories are listed:

evaluation number	test result	deviation from assigned value	Z-Score Horwitz	Z'-Score Horwitz	Remarks

4.1 Melamine (in mg/kg)

Statistic Data	
Number of results	*9*
Number of outliers	*0*
Mean	19,8
Median	18,8
Robust mean (X)	19,9
Robust standard deviation (S^x)	7,96
Target range:	
Target standard deviation $\hat{\sigma}'$	3,89
lower limit of target range *	12,1
upper limit of target range *	27,7
Quotient $S^x/\hat{\sigma}'$	*2,0*
Standard uncertainty u_X	*3,32*
Quotient $u_X/\hat{\sigma}'$	*0,85*
Number of results in the target range	*6 (67%)*

** target range calculated from z'-score*

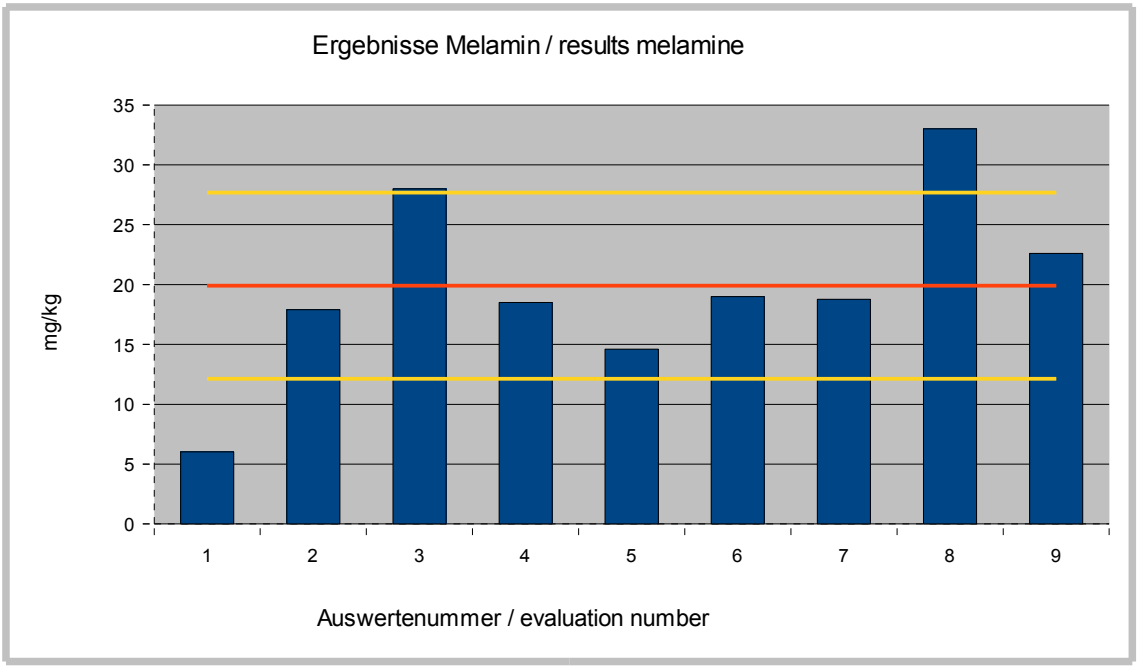


Fig. 1: Results Melamine
(red line = robust mean, yellow lines = target range)

Results of participants

Evaluation number	Result	Deviation	Z-Score (for Info)	Z'-Score	Remarks
	[mg/kg]	X rob. Mean	Horwitz	Horwitz	
1	6,033	-13,876	-6,8	-3,6	
2	17,9	-2,009	-1,0	-0,5	
3	28	8,091	4,0	2,1	
4	18,5	-1,409	-0,7	-0,4	
5	14,6	-5,309	-2,6	-1,4	
6	18,99	-0,919	-0,5	-0,2	
7	18,77	-1,139	-0,6	-0,3	
8	33	13,091	6,4	3,4	
9	22,6	2,691	1,3	0,7	

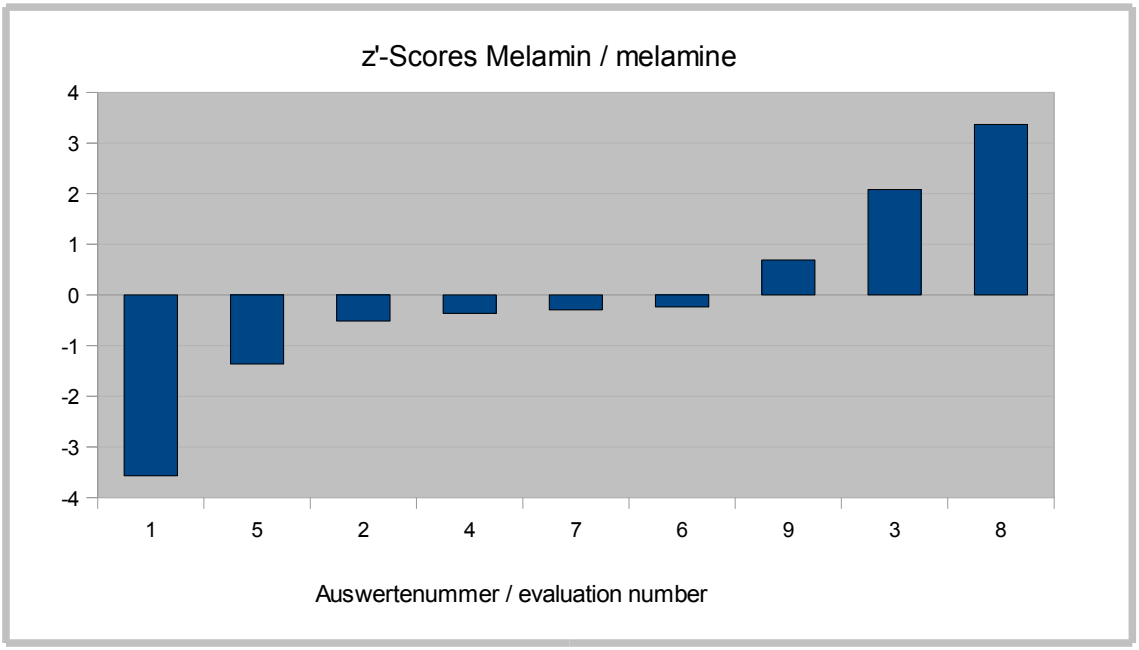


Fig. 2: Z'-Scores Melamine

5. Documentation

5.1 Primary data

5.1.1 Melamine

Evaluation number	Result	Sample-No. A	Sample No. B	Result A	Result B	Recovery Rate
	[g/100g]			g/100g	g/100g	%
1	6,033	8	36	6.747	6.670	111,2
2	17,9	3; 50	3; 50	17,6	18,2	100
3	28			28	29	
4	18,5	35	48	18,0	19,0	96
5	14,6	32	45	14,9	14,2	-
6	18,99	16; 28	16;28	17,39	20,58	
7	18,77	11	24	19,59	17,96	72
8	33	1; 23	1; 23	33,09	32,91	69,89
9	22,6	19; 30	19; 30	21,2	23,9	

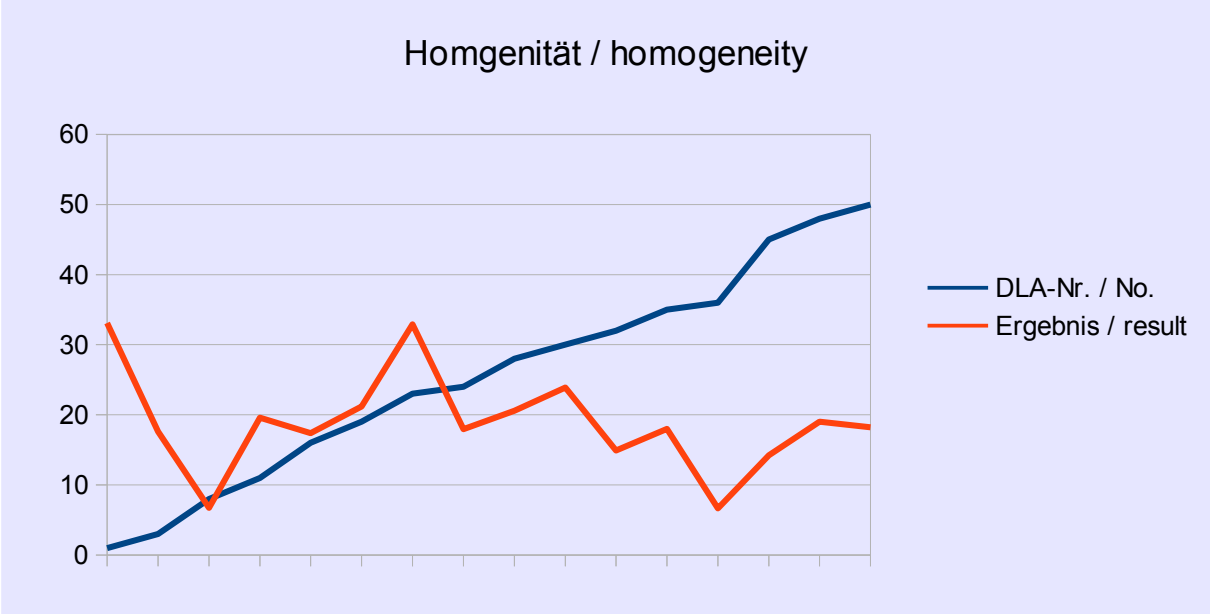
5.2 Homogeneity

5.2.1 Repeatability standard deviation of duplicate tests of the participants

The repeatability standard deviation was calculated with the data documented in 5.1.3 for melamine. It is 1,52 mg/kg = 7,6 % of X.

5.2.2 Comparison of sample number / test result

The comparison of the increasing sample-numbers and measured results could show a sufficient homogeneity. The trend line for the results showed a slitghly negative slope of 0,4 mg/kg. This was respected by evaluating the results using the z'score.



5.3 Analytical Methods

Details by the participants

5.3.1 Melamine

Evaluation number	Method description and further remarks	Recovery with same matrix	Accredited	Remarks
1	ELISA test, Max signal - Melamine elia Test Kit - reference 1077-01	yes	yes	Results are expressed with a dot as decimal separator
2	SFS05- Melamine in foods by LC-MS/MS, (Extraction with methanol-water / acetonitril) (against isotop-marked melamine)	yes	yes	Recovery 100%, due to quantification against isotop-marked melamine; sample "A" labelled: "Sample 50"; sample "B" labelled "Sample03"
3	FDA, adapted		no	
4	according to FDA LIB NR 4421	yes	yes	Problems with homogeneity A/B
5	ELISA-Test	no	yes	
6	LC-MS/MS	no	no	
7	in house method	yes	yes	
8	ELISA	yes	no	-
9	LC-MS/MS	yes	yes	recovery 100 % due to ISTD

6. Index of participant laboratories

Teilnehmer / Participant	Ort / Town	Land / Country
		GERMANY
		GERMANY
		GERMANY
		GERMANY
		BELGIUM
		GERMANY
		GERMANY
		NETHERLANDS
		SERBIA
		GERMANY

[The address data of the participants were deleted for publication of the evaluation report.]

7. Index of references

1. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
2. Verordnung / Regulation 882/2004/EU; Verordnung über amtliche Kontrollen / Regulation on official controls
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